AN 10/029,840
GAU 1648

PCT

WORLD INTELLECTUAL PROPERTY ORGANIZATION
International Bureau

INTERNATIONAL APPLICATION PUBLISHED UNDER THE PATENT COOPERATION TREATY (PCT)

(51) International Patent Classificati n 6 : C12Q | A2

(11) International Publication Number: WO 99/04029

(43) International Publication Date: 28 January 1999 (28.01.99)

(21) International Application Number: PCT/US98/14665

(22) International Filing Date: 17 July 1998 (17.07.98)

(30) Priority Data:
60/053,069 18 July 1997 (18.07.97) US

(63) Related by Continuation (CON) or Continuation-in-Part (CIP) to Earlier Application
US 60/053,069 (CIP)
Filed on 18 July 1997 (18.07.97)

(71) Applicant (*for all designated States except US*): THE GOVERNMENT OF THE UNITED STATES OF AMERICA, represented by THE SECRETARY, DEPARTMENT OF HEALTH AND HUMAN SERVICES [US/US]; Office of Technology Transfer, National Institutes of Health, Suite 325, 6011 Executive Boulevard, Rockville, MD 20852 (US).

(72) Inventors; and
(75) Inventors/Applicants (*for US only*): MENG, Xiang-Jin [CN/US]; Apartment 611, 3 Pooks Hill Road, Bethesda, MD 20814 (US). EMERSON, Suzanne, U. [US/US]; 18201 Woodcrest Drive, Rockville, MD 20852 (US). PURCELL, Robert, H. [US/US]; 17517 White Grounds Road, Boyds, MD 28841 (US).

(74) Agents: FEILER, William, S. et al.; Morgan & Finnegan, L.L.P., 345 Park Avenue, New York, NY 10154 (US).

(81) Designated States: AL, AM, AT, AU, AZ, BA, BB, BG, BR, BY, CA, CH, CN, CU, CZ, DE, DK, EE, ES, FI, GB, GE, GH, GM, HR, HU, ID, IL, IS, JP, KE, KG, KP, KR, KZ, LC, LK, LR, LS, LT, LU, LV, MD, MG, MK, MN, MW, MX, NO, NZ, PL, PT, RO, RU, SD, SE, SG, SI, SK, SL, TJ, TM, TR, TT, UA, UG, US, UZ, VN, YU, ZW, ARIPO patent (GH, GM, KE, LS, MW, SD, SZ, UG, ZW), Eurasian patent (AM, AZ, BY, KG, KZ, MD, RU, TJ, TM), European patent (AT, BE, CH, CY, DE, DK, ES, FI, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE), OAPI patent (BF, BJ, CF, CG, CI, CM, GA, GN, GW, ML, MR, NE, SN, TD, TG).

Published
Without international search report and to be republished upon receipt of that report.

(54) Title: A SWINE HEPATITIS E VIRUS AND USES THEREOF

(57) Abstract

The present invention discloses the isolation and characterization of a novel swine hepatitis E virus and the use of the virus, the proteins and its nucleic acid sequence as diagnostic reagents and vaccines.

FOR THE PURPOSES OF INFORMATION ONLY

Codes used to identify States party to the PCT on the front pages of pamphlets publishing international applications under the PCT.

AL	Albania	ES	Spain	LS	Lesotho	SI	Slovenia
AM	Armenia	FI	Finland	LT	Lithuania	SK	Slovakia
AT	Austria	FR	France	LU	Luxembourg	SN	Senegal
AU	Australia	GA	Gabon	LV	Latvia	SZ	Swaziland
AZ	Azerbaijan	GB	United Kingdom	MC	Monaco	TD	Chad
BA	Bosnia and Herzegovina	GE	Georgia	MD	Republic of Moldova	TG	Togo
BB	Barbados	GH	Ghana	MG	Madagascar	TJ	Tajikistan
BE	Belgium	GN	Guinea	MK	The former Yugoslav Republic of Macedonia	TM	Turkmenistan
BF	Burkina Faso	GR	Greece			TR	Turkey
BG	Bulgaria	HU	Hungary	ML	Mali	TT	Trinidad and Tobago
BJ	Benin	IE	Ireland	MN	Mongolia	UA	Ukraine
BR	Brazil	IL	Israel	MR	Mauritania	UG	Uganda
BY	Belarus	IS	Iceland	MW	Malawi	US	United States of America
CA	Canada	IT	Italy	MX	Mexico	UZ	Uzbekistan
CF	Central African Republic	JP	Japan	NE	Niger	VN	Viet Nam
CG	Congo	KE	Kenya	NL	Netherlands	YU	Yugoslavia
CH	Switzerland	KG	Kyrgyzstan	NO	Norway	ZW	Zimbabwe
CI	Côte d'Ivoire	KP	Democratic People's Republic of Korea	NZ	New Zealand		
CM	Cameroon			PL	Poland		
CN	China	KR	Republic of Korea	PT	Portugal		
CU	Cuba	KZ	Kazakstan	RO	Romania		
CZ	Czech Republic	LC	Saint Lucia	RU	Russian Federation		
DE	Germany	LI	Liechtenstein	SD	Sudan		
DK	Denmark	LK	Sri Lanka	SE	Sweden		
EE	Estonia	LR	Liberia	SG	Singapore		

A SWINE HEPATITIS E VIRUS AND USES THEREOF

FIELD OF THE INVENTION

Hepatitis E occurs predominantly in developing countries of Asia and Africa but has also been the cause of epidemics in Mexico (1). The disease generally affects young adults and has a very high mortality rate, up to 20%, in pregnant women (1-4). Hepatitis E has rarely been reported in developed countries, and most of those cases have been imported (1, 4-6). The causative agent, hepatitis E virus (HEV), is transmitted primarily by the fecal-oral route, often through contaminated water (1, 4). The availability of sensitive serological tests for HEV has permitted detailed assessment of the prevalence of HEV infection (7-8). In regions where HEV is endemic, anti-HEV antibodies have been detected in sera from convalescent individuals as well as from the general population (1, 3). Although hepatitis E is not endemic in the United States and other developed countries, anti-HEV was found in a significant proportion, up to 28% in some areas, of healthy individuals in these countries (7, 9). It is unclear if the anti-HEV detected in developed countries demonstrates infection with a non-pathogenic HEV strain or cross-reactivity with a related agent.

It has been reported that anti-HEV is acquired naturally in primates and swine (1, 10), suggesting that these species have been exposed to HEV or a related agent, and that hepatitis E may be a zoonotic disease. The role of swine in HEV transmission is not clear although

domestic swine have been reported to be susceptible to infection with a human HEV strain (11). It would be advantageous to have an animal strain of HEV that could be studied in animals but would sufficiently resemble the human virus as to make the results of studies with the animal virus applicable to the human disease. It would be particularly advantageous to have an animal virus that could be useful in preparing vaccines and other medicaments for treatment of animals, especially humans.

BACKGROUND OF THE INVENTION

It has been reported that domestic swine can be experimentally infected with HEV (11), although this infection appears more severe in swine than in non-human primates. In our own examination, swine inoculated with human HEV remained clinically normal and we detected no antibody response to HEV. While it has been suggested that human feces from infected individuals are the primary source of infection for hepatitis E in humans, swine have been suggested as a possible animal reservoir and natural host for the virus. For example, in some geographical areas the prevalence of anti-HEV in swine is even higher than that found in humans and swine have been suggested as a possible year round reservoir for HEV (10). However, the occurrence of IgG anti-HEV in swine does not necessarily mean that HEV infection has occurred. Hepatitis E is not endemic in the United States and yet our own studies have indicated that the majority of swine 2 to 3 months of age in the midwestern United States have IgG anti-HEV. In addition, IgG anti-HEV is also found in a significant proportion of healthy humans in countries, including the United States, where hepatitis E is not

endemic (7). Such data indicated to us that an agent or agents other than human HEV, but antigenically closely related to human HEV, exists within the swine population. The antibody induced by this putative agent has been shown to cross-react with a human HEV antigen used in serological testing. Such findings mean that caution must be used in interpreting serological data, especially from non-endemic regions. Identification of such a putative agent would, of course, be highly advantageous in order to develop more specific serological tests for HEV infection. The detection and isolation of such a virus from swine would also be desirable in determining the possible existence of an animal reservoir for HEV. In addition, such a virus, if capable of infecting swine but not causing illness, might be well suited for use as a vaccine or therapeutic agent for use in vaccinating and treating mammals, especially humans. Thus, such a virus could well serve as an attenuated live virus vaccine strain.

BRIEF SUMMARY OF THE INVENTION

The present invention is directed to a novel swine hepatitis E virus (swine HEV) strain in isolated form and with sufficient nucleotide sequence homology in its genome, and sufficient amino acid sequence homology in its capsid protein, both relative to human HEV, as to be highly useful in the evaluation of potential human infection by endogenous swine sources, including potential infection resulting from xenotransplantation, especially transplanting of swine organs and tissues into humans.

The swine hepatitis E virus of the present invention is also useful as a vaccine for vaccination of animals, preferably mammals, and most preferably humans,

against infection by other strains of hepatitis E virus. The swine HEV of the present invention would, for example, be especially useful as a vaccine for use in humans to prevent possible infection by human hepatitis E virus (or HEV). The swine HEV of the present invention accomplishes this by providing only a subclinical infection on its own and thereby immunizing the subject animal, for example, a human, in, for example, a manner similar to that accomplished by cowpox virus in immunizing against smallpox.

The present invention also relates to use of a novel swine HEV as a therapeutic treatment for infection by other strains of HEV by injection of the virus of the invention to bolster the immune response of an infected animal while providing only a subclinical infection on its own.

The swine HEV of the present invention is also highly useful in the generation of both polyclonal and monoclonal antibodies which themselves find use as therapeutic agents for treatment of animals, especially mammals, and most especially humans, in need thereof.

The antibodies produced in response to the swine HEV of the present invention also find use in the development of *in vitro* diagnostic protocols for early detection of HEV infection in animals, especially mammals, and most especially humans.

The swine HEV of the present invention is particularly advantageous for use in the development of prophylactic, therapeutic and diagnostic agents for the prevention, treatment and detection of human HEV because it is not a human virus and thus can be handled both

experimentally and clinically without fear of severe infection and/or contamination.

SPF swine were experimentally infected with the swine HEV of the present invention. Therefore, infection of swine with the swine HEV can provide an appropriate animal model for human HEV experiments.

BRIEF DESCRIPTION OF THE DRAWINGS

Figure 1. This figure shows seroconversion of 3 representative piglets to anti-HEV by plotting age (abscissa) in weeks versus the absorbance (or OD) of serum for IgG (left ordinate) and IgM (right ordinate); (A) a piglet born to a seropositive sow with a high titer of IgG anti-HEV; (B) a piglet born to a seropositive sow with a lower titer of IgG anti-HEV; (C) a piglet born to a seronegative sow. The ELISA-absorbance value of IgG anti-HEV in breeder sows is shown at (S).

Figure 2. Amplification of swine HEV-specific fragment by RT-PCR. Serum samples from 2 piglets (#4 and #14) obtained 1 week before (-1) and the week of (0) seroconversion were used for RT-PCR of a 344 bp fragment. Serum samples obtained at the same time (weeks 19 and 20) after birth from a seronegative piglet (#15) were also included. "L" represents the molecular weight markers.

Figure 3. Alignment of amino acid sequences of Open Reading Frames 2 (orf 2) (at A) and orf 3 (at B) of swine HEV with human strains of HEV. The sequence of the Sar55 strain is shown at the top, and only differences between them are indicated. Deletions are indicated by (-). The putative hypervariable region (HVR) in the ORF3 is indicated by asterisks (*). The sequences used in this

alignment were Burma (14), Mexico (15) NE8L (Myanmar, 16), Hyderabad (India, 17), Madras (India, GenBank Accession No. 99441), HEV037 (isolate from a case of fulminant hepatitis, GenBank Accession No. X98292), Sar55 (Pakistan, 18), KS2-87 (China, 19), Hetian (China, GenBank Accession No. L08816), Uigh179 (China, 20).

Figure 4. Phylogenetic tree based on the complete nucleotide sequences of ORFs2 and 3. The tree was constructed by maximum parsimony methods with the aid of PAUP software package version 3.1.1. The tree with the shortest length (most parsimonious) was found by implementing the bootstrap (1000 replicas) using the branch-and-bound search option. The branch lengths (number given above each branch) are indicated.

Figure 5. Experimental infection of specific-pathogen-free swine with the swine HEV. The IgG anti-HEV response is plotted. Viremia was measured by RT-PCR as indicated along the top.

Figures 6A-6J show the nucleotide (Figs. 6A-6C)and deduced amino acid sequences (Figs. 6D-6J) of the ORF1 of the swine HEV.

Figures 7A-7D show the complete genomic sequence of the swine HEV.

Figure 8. Amplification of the complete genome of swine HEV. **(A)** The genomic organization of HEV and its putative functional domains are shown beneath the nucleotide scale bar (in Kb). The relative positions of the PCR fragments are indicated by bars. **(B)** Oligonucleotide primers used to amplify each fragment are indicated. Both the first round PCR primers and the second round nested PCR primers (*italics*) are shown.

Degenerate bases are shown in parentheses. Mt, methyltransferase; Y, Y domain; P, cysteine-like protease; Pro, proline-rich "hinge" region; X, X domain; Hel, helicase; RDRP, RNA-directed RNA polymerase.

DETAILED DESCRIPTION OF THE INVENTION

The present invention relates to novel and useful hepatitis E virus strains from swine and naturally occurring mutants thereof.

As shown a long time ago with smallpox virus, it is highly advantageous to have available an attenuated virus, such as cowpox, to use for vaccination purposes. Such relatively innocuous virus particles, which can cross-react with antibodies against the more deadly organisms, can often be used to produce a highly effective vaccine to immunize at-risk animals, especially humans, against infection with otherwise extremely virulent microbes. In the same way, it would behighly advantageous to have a similar attenuated type of vaccine to use in immunization programs against hepatitis E virus. It is an object of this invention to provide such a vaccine.

In accordance with the present invention, a swine hepatitis E virus has been identified and shown to be similar in its characteristic properties to human hepatitis E virus. The virus of the present invention has been shown to be similar to the human virus by demonstrating seroconversion of pigs to anti-HEV, sequence similarity between the virus of the invention and human strains of HEV, viremia just prior to seroconversion, and histologic evidence of hepatitis infection in naturally-infected pigs. The swine-HEV cross-reacted with human HEV

capsid antigen, and the infected piglets showed microscopic evidence of hepatitis during the acute stage of the infection.

The high prevalence of anti-HEV in commercial swine herds suggest that swine HEV is widespread in the general swine population. However, our results showed that naturally infected young pigs did not display clinical symptoms despite microscopic evidence of hepatitis. This suggests that swine HEV causes only subclinical infection in young pigs, a situation reminiscent of hepatitis A virus (HAV) in humans (21). Children infected with HAV are often asymptomatic, but most adults infected show typical clinical symptoms(21). It is difficult to evaluate the outcome of natural swine HEV infection in adult pigs since virtually all swine at least 3 months of age had IgG anti-HEV. Experimental infection of adult SPF swine with swine HEV will likely be necessary to answer this question.

The amino acid differences between swine and human HEV in the putative capsid gene are less than 10%. However, the high degree of amino acid sequence conservation in the capsid gene among human strains of HEV could indicate a functional significance for the differences between swine and human HEV. For example, in some cases only a few changes, or even a single change, in amino acid units of a structural protein have dramatically altered viral tropism and pathogenicity (22, 23). Of course, it is not clear whether swine HEV evolved into human HEV, or vice versa, or whether they diverged from a common ancestor. Regardless of lineage, the possibility that swine HEV could infect humans raises a potential public health concern for zoonosis or xenozoonosis, especially since xenotransplantation of pig organs has

been suggested as a solution to the solid organ donor shortage for transplantations. Thus, xenozoonoses, the inadvertent transmission of pathogens from animal organs to human recipients, is of major concern (24). Viruses pathogenic for pigs might pose a risk to humans. However, nonpathogenic pig viruses may also become pathogenic for humans after xenotransplantation, as a result of species-jumping, recombination or adaptation in immunocompromised xenotransplantation recipients (24). Furthermore, pigs recovered from swine HEV infection might have a damaged liver (or other organ) which would limit usefulness for xenotransplantation.

Because of these and other potential public health concerns, it would be highly advantageous to have a swine HEV that is sufficiently closely related to human HEV as to allow evaluation as a potential source of infection in humans. In accordance with the present invention, such a swine HEV has been isolated and shown to have substantial nucleotide sequence homology with the human hepatitis E virus. Also in accordance with the present invention, the entire open reading frames (ORF) 2 and 3 were amplified by RT-PCR from sera of naturally-infected pigs. The putative capsid gene (ORF2) of swine HEV shares about 79% to about 80% sequence identity at the nucleotide level and about 90% to about 92% identity at the amino acid level related to human HEV strains. The small ORF3 of swine HEV had about 83% to about 85% nucleotide sequence identity and about 77% to about 82% amino acid identity with human HEV strains. The putative nonstructural proteins of ORF1 of swine HEV share about 98% amino acid identity with the U.S. human isolates of HEV but only about 80-81% identity with HEV strains from

Asia and Mexico. Phylogenetic analyses showed that swine HEV is closely related to, but distinct from, human HEV strains.

The availability of an HEV capable of infecting both humans and animals with a subclinical hepatitis infection would be highly advantageous if used prophylactically, in the form of a vaccine, or therapeutically, as an inoculum to treat early-stage infections. When the present invention is used prophylactically, the agents are provided in advance of any symptom. This prophylactic use of the swine HEV serves to prevent or ameliorate any subsequent infection. When used therapeutically, the swine HEV of the present invention is provided at (or shortly after) the onset of any symptoms of infection. The swine HEV of the present invention can therefore be provided either before any anticipated exposure to hepatitis E virus (either swine or human), so as to ameliorate the anticipated severity, duration or extent of any subsequent infection or disease conditions, or after the onset of infection and disease.

When the swine HEV of the present invention is used as a vaccine, said vaccine comprises a pharmaceutical composition containing a sufficient number of viral particles, or recombinant proteins, to elicit a prophylactically effective immune response in the organism to be vaccinated, said amount also depending on the route of administration. The vaccine according to the present invention can thus be administered by oral, subcutaneous, intravenous, intramuscular or intraperitoneal routes. One skilled in the art will certainly appreciate that the amounts to be administered for any particular treatment protocol can be readily determined without undue

experimentation. Suitable amounts might be expected to fall within the range of about 2 μg of viral protein per kg of body weight to about 100 μg viral protein per kg of body weight. Of course, the actual amounts will vary depending on the route of administration as well as the sex, age, and clinical status of the subject which, in the case of human patients, is to be determined within the sound judgment of the clinician.

The vaccine of the present invention may be employed in such forms as capsules, liquid solutions, suspensions or elixirs for oral administration, or sterile liquid forms such as solutions or suspensions. Any inert carrier is preferably used, such as saline, phosphate-buffered saline, or any such carrier in which the swine HEV of the present invention can be suitably suspended. The vaccines may be in the form of single dose preparations or in multi-dose flasks which can be utilized for mass-vaccination programs of both animals and humans. For purposes of using the swine HEV of the present invention as a vaccine, reference is made to Remington's *Pharmaceutical Sciences*, Mack Publishing Co., Easton, Pa., Osol (Ed.) (1980); and *New Trends and Developments in Vaccines*, Voller et al (Eds.), University Park Press, Baltimore, Md. (1978), both of which provide much useful information for preparing and using vaccines. Of course, the swine HEV, when used as a vaccine, can include, as part of the composition or emulsion, a suitable adjuvant, such as alum (or aluminum hydroxide) when humans are to be vaccinated, to further stimulate production of antibodies by immune cells.

When the swine HEV of the present invention is used as a vaccine, the virus itself may be live, killed,

or live but attenuated. However, with a swine HEV producing only subclinical symptoms in swine and in other animals, especially humans, further attenuation may not be necessary.

When the swine HEV of the present invention is used as a vaccine or inoculum, it will normally exist as a physically discrete unit suitable as a unitary dosage for animals, especially mammals, and most especially humans, wherein each unit will contain a predetermined quantity of active viral material calculated to produce the desired immunogenic effect in association with the required diluent. The dose of said vaccine or inoculum according to the present invention is administered at least once. In order to increase the antibody level, a second or booster dose may be administered at some time after the initial dose. The need for, and timing of, such booster dose will, of course, be determined within the sound judgment of the administrator of such vaccine or inoculum and according to sound principles well known in the art. For example, such booster dose could reasonably be expected to be advantageous at some time between about 2 weeks to about 6 weeks following the initial vaccination. Subsequent doses may be administered as indicated.

The swine HEV of the present invention can also be administered for purposes of therapy, where an animal, especially a mammal, and most especially a human, is already infected, as shown by well known diagnostic measures. When the swine HEV of the present invention is used for such therapeutic purposes, much of the same criteria will apply as when it is used as a vaccine, except that inoculation will occur post-infection. Thus, when the swine HEV of the present invention is used as a

therapeutic agent in the treatment of infection said therapeutic agent comprises a pharmaceutical composition containing a sufficient number of viral particles to elicit a therapeutically effective response in the organism to be treated, said amount also depending on the route of administration. The therapeutic agent according to the present invention can thus be administered by oral, subcutaneous, intravenous, intramuscular or intraperitoneal routes, with oral or intravenous routes being preferred. One skilled in the art will certainly appreciate that the amounts to be administered for any particular treatment protocol can be readily determined without undue experimentation. Suitable amounts might be expected to fall within the range of about 2 μg of viral protein per kg of body weight to about 100 μg viral protein per kg of body weight. Of course, the actual amounts will vary depending on the route of administration as well as the sex, age, and clinical status of the subject which, in the case of human patients, is to be determined within the sound judgment of the clinician.

The therapeutic agent of the present invention can be employed in such forms as capsules, liquid solutions, suspensions or elixirs for oral administration, or sterile liquid forms such as solutions or suspensions. Any inert carrier is preferably used, such as saline, phosphate-buffered saline, or any such carrier in which the swine HEV of the present invention can be suitably suspended. The therapeutic agents may be in the form of single dose preparations or in the multi-dose flasks which can be utilized for mass-treatment programs of both animals and humans. Of course, when the swine HEV of the present invention is used as a therapeutic agent it may be

administered as a single dose or as a series of doses, depending on the situation as determined by the person conducting the treatment.

The swine HEV of the present invention can also be utilized in the production of antibodies against HEV. The term "antibody" is herein used to refer to immunoglobulin molecules and immunologically active portions of immunoglobulin molecules. Examples of antibody molecules are intact immunoglobulin molecules, substantially intact immunoglobulin molecules and portions of an immunoglobulin molecule, including those portions known in the art as Fab, $F(ab')_2$ and F(v) as well as chimeric antibody molecules.

The swine HEV of the present invention can be used in the generation of antibodies that immunoreact (i.e., specific binding between an antigenic determinant-containing molecule and a molecule containing an antibody combining site such as a whole antibody molecule or an active portion thereof) with antigenic determinants on the surface of hepatitis E virus particles that commonly infect non-swine species, especially humans. Thus, the observed cross-reaction between the swine HEV of the present invention and antibodies against human HEV make the swine HEV useful in generation of antibodies against human HEV, thus facilitating the use of the swine HEV as a vaccine for humans.

The present invention also relates to antibodies produced following immunization with the swine HEV according to the present invention. These antibodies are typically produced by immunizing a mammal with an immunogen or vaccine comprising the swine HEV of the

present invention to induce, in said mammal, antibody molecules having immunospecificity for the swine HEV. When used in generating such antibodies, the swine HEV of the present invention may be linked to some type of carrier molecule. The resulting antibody molecules are then collected from said mammal.

The antibody molecules of the present invention may be polyclonal or monoclonal. Monoclonal antibodies are readily produced by methods well known in the art. Portions of immunoglobulin molecules, such as Fabs, as well as chimeric antibodies may also be produced by methods well known to those of ordinary skill in the art of generating such antibodies and requires no specific elucidation herein.

The antibody according to the present invention may be contained in blood plasma, serum, hybridoma supernatants, and the like. Alternatively, the antibody of the present invention is isolated to the extent desired by well known techniques such as, for example, using DEAE Sephadex. The antibodies produced according to the present invention may be further purified so as to obtain specific classes or subclasses of antibody such as IgM, IgG, IgA, and the like. Antibodies of the IgG class are preferred for purposes of passive protection.

The antibodies of the present invention are useful in the prevention and treatment of diseases caused by hepatitis E virus in animals, especially mammals, and most especially humans.

In providing the antibodies of the present invention to a recipient mammal, preferably a human, the dosage of administered antibodies will vary depending on

such factors as the mammal's age, weight, height, sex, general medical condition, previous medical history, and the like.

In general, it will be advantageous to provide the recipient mammal with a dosage of antibodies in the range of from about 1 mg/kg body weight to about 10 mg/kg body weight of the mammal, although a lower or higher dose may be administered if found desirable. Such antibodies will normally be administered by intravenous route as an inoculum. The antibodies of the present invention are intended to be provided to the recipient subject in an amount sufficient to prevent, lessen or attenuate the severity, extent or duration of any existing infection.

The antibodies prepared by use of the swine HEV of the present invention are also highly useful for diagnostic purposes. The antibodies can be used as *in vitro* diagnostic agents to test for the presence of virus in biological samples taken from animals, especially swine and humans. Such assays include, but are not limited to, radioimmunoassays, EIA, fluorescence, Western blot analysis and the like. In one such embodiment, the biological sample is contacted with antibodies of the present invention and a labeled second antibody is used to detect the presence of HEV to which the antibodies are bound.

Such assays may be, for example, of direct protocol (where the labeled first antibody is immunoreactive with the antigen, such as, for example, a protein on the surface of the virus), an indirect protocol (where a labeled second antibody is reactive with the first antibody), a competitive protocol (such as would

involve the addition of a labeled antigen), or a sandwich protocol (where both labeled and unlabeled antibody are used), as well as other protocols well known and described in the art.

In one embodiment, an immunoassay method would utilize an antibody specific for a substance comprising HEV surface antigen (HCSA) determinants and would further comprise the steps of contacting a test sample containing said test substance containing the HESA determinants with the HEV-specific antibody and then detecting the presence of HEV material in the test substance using one of the types of assay protocols as described above.

Recombinant proteins and antibodies produced according to the present invention may also be supplied in the form of a kit, either present in vials as purified material, or present in compositions and suspended in suitable diluents as previously described. In a preferred embodiment, such a diagnostic test kit for detection of HEV antigens in a test sample comprises in combination a series of containers, each container a reagent needed for such assay. Thus, one such container would contain a specific amount of HEV-specific antibody as already described, a second container would contain a diluent for suspension of the sample to be tested, a third container would contain a positive control and an additional container would contain a negative control. An additional container could contain a blank.

Because the Open Reading Frame (ORF2) that codes for a putative capsid protein has been cloned *in vitro* it can be used to prepare recombinant proteins that themselves are available for use as vaccines and

therapeutic agents. In accordance with the present invention, said capsid protein antigen has been prepared. A similar approach is advantageous with respect to ORFs 1 and 3 and their protein products. Since such recombinant proteins also represent antigenic determinants on the virus, and presumably are responsible for the immunogenicity of the particles of the swine hepatitis E virus of the present invention, such recombinant proteins can also be used to generate antibodies useful as therapeutic agents in the treatment of hepatitis E in animals, especially humans. Such antibodies can also be used in protocols for *in vitro* assays for detection of hepatitis E virus in an animal, especially a human, suspected of being infected therewith.

Because the swine HEV can be used to infect cells in culture, the present invention also relates to a method for determining the susceptibility of cells *in vitro* to support HEV infection. This would permit determination of the susceptibility of cells from various organs, for example, of the pig, to determine their potential use for possible transplantation into other animals, especially humans. Cells from organs susceptible to such infection might be questionable candidates for eventual transplantation. In one such embodiment, the method would comprise the growing of animal cells, especially swine cells, in vitro and exposing said cells to the swine HEV of the present invention, then determining if the cells show indicia of HEV replication. Such indicia would include the detection of viral antigens in the cell, for example, by immunofluorescent procedures well known in the art. Such viral proteins would also be detected by Western blotting using antibodies specific

therefor. Such indicia would also include the successful extraction of newly transcribed viral DNA within the cells. The presence of live, infectious virus particles following such test could also be shown by injection of cell culture medium into healthy animals, with subsequent exhibition of the symptoms of HCV infection. Such testing could also be carried out with cells from tissues of other species to determine their susceptibility to infection by the swine HEV of the present invention, relying on the same criteria to show viral replication.

It is known that swine show antibodies to HEV and have been suggested as a possible animal reservoir for this virus. In addition, IgG anti-HEV is also found in a significant proportion of otherwise healthy humans from different areas. Since the causative agent is still unclear, it would be highly advantageous to determine if the antibodies found in an animal, especially a human, are the result of human HEV or perhaps a swine HEV or other animal. It is thus an object of the present invention to provide a method for differential diagnosis of HEV antibodies in animals, especially in humans, to determine the likely causative agent thereof and thus the presence of possible subclinical infections that can serve as a source of the disease. Armed with the virus of the present invention many protocols for such diagnoses will no doubt suggest themselves to those of skill in the art.

In one embodiment of the present invention a tissue sample from an animal, perhaps one suspected of harboring an infection of HEV, will be obtained and then treated with antibodies specific for either human HEV antigenic determinants or the swine HEV antigenic determinants, such as an antibody of the present

invention. The formation and detection of a complex between the substance in the sample that possesses such determinants and the antibodies specific for the virus will indicate the presence of viral determinants. By titering the different antibodies to determine their relative occurrence, it is then possible to determine if the source of the antigen is of human or swine, or other animal, origin. Such differential diagnosis can be carried out on any animal, especially primates and other mammals, and most especially humans. The sample to be tested can, of course, be any tissue excised from said animal, especially blood and other fluids.

Today there is a great thrust toward the use of animal organs for transplantation into other animals, especially into humans, with pigs as a suggested and useful donor organism. Before such programs can continue, or even begin, it would be highly advantageous to determine if any infectious agents exist in the donor animal and, if so, whether such organisms can infect the donee, especially humans. Because IgG anti-HEV is found in swine and the swine HEV of the present invention has been recovered from pigs and has great similarity to the human HEV, it is a potentially infectious agent in humans. It is thus an object of the present invention to utilize the swine HEV as a test organism for the assessment of risk factors in such xenotransplantation between the pig, as donor, and other animals, especially humans.

Various protocols to test transplantable organs and tissues for such potentially infectious agents will, of course, suggest themselves to those of skill in the art. In one such embodiment of the present invention, a biological sample of a tissue, for example, from a pig to

be used as a donor organism, will be obtained prior to any transplantation and such sample is then tested for the presence of swine HEV. Such tissues for transplantation may include liver, heart and kidneys, but are by no means limited to these. The testing of such tissue for the presence of swine HEV can be accomplished by a number of procedures already disclosed herein and using methods well known in the art. For example, a candidate tissue can be examined using antibodies tagged with a label, such as an immunofluorescent label, to determine the presence or absence of swine HEV antigenic determinants therein, with complex formation (a positive reaction) indicating the presence of viral contamination. In addition, DNA extracted from cells of the candidate tissue can be amplified by RT-PCR (reverse transcriptase-polymerase chain reaction) and tested for hybridization therewith using nucleic acid probes, especially chemically or radioactively labeled probes, to determine the presence or absence of sequences unique to the swine HEV genome where hybridization indicates the presence of viral contamination.

For all prophylactic, therapeutic and diagnostic uses, the swine HEV of the present invention, alone or linked to some carrier, as well as antibodies and other reagents, plus appropriate devices and accessories, may be provided in the form of a kit so as to facilitate ready availability and ease of use.

MATERIALS AND METHODS

Serum samples. Serum samples were obtained from swine of various ages in 15 commercial herds and one specific-pathogen-free (SPF) herd in the midwestern United

States.

Table 1. IgG anti-HEV prevalence in swine from commercial herds in the United States.

Herd	Age	No. of swine tested	No. of swine with anti-HEV(%)
A	6 wk	8	0(0)
	12 wk	8	0(0)
	20 wk	8	8(100)
	26 wk	8	5(63)
	Adult	25	14(56)
B	3-4 wk	8	0(0)
	5-6 wk	8	0(0)
	7-8 wk	8	0(0)
	13 wk	12	10(83)
	6 mo	8	8(100)
	Adult	17	16(94)
C	2 mo	8	1(13)
	3 mo	8	8(100)
	4 mo	8	4(50)
	5 mo	8	8(100)
	Adult	8	8(100)
D	2 mo	10	2(20)
E	6 mo	10	10(100)
F	6 mo	10	10(100)
G	6 mo	10	10(100)
H	8 mo	10	10(100)
I	>1 yr	10	10(100)
J	1-2 yr	10	10(100)
K	1-3 yr	10	10(100)
L	2 yr	10	10(100)
M	2-3 yr	10	10(100)
N	Adult	19	15(79)
O	Adult	6	5(83)
P*	Adult	10	0(0)

*Specific-pathogen-free (SPF) swine herd.

Preparation of HEV putative capsid antigen. Insect cells were infected with recombinant baculovirus containing the putative capsid gene (ORF2) sequence of a Pakistani strain of HEV, SAR55 (8). A 55 kD recombinant protein expressed from a recombinant baculovirus containing ORF2 was purified from insect cells (8) and used for the standard ELISA. The ORF2 recombinant protein was further purified by high pressure liquid chromatography(HPLC).

Generation of hyperimmune swine antibody to HEV. Two SPF swine, 3 weeks old, were immunized intramuscularly with 50 µg of HPLC-purified ORF2 recombinant protein mixed with Freund's incomplete adjuvant. Booster immunizations were given at 2 and 4 weeks after the first immunization. Sera obtained before immunization and weekly for 9 weeks after immunization were used to develop an ELISA (see below).

ELISA for anti-HEV in swine. The standard ELISA for anti-HEV in swine was performed essentially as described for anti-HEV in chimpanzees (8, 12), except that the secondary antibody was replaced with peroxidase-labeled goat anti-swine IgG (KPL, Gaithersburg, MD). All of the swine serum samples were tested in duplicate. Preimmune and hyperimmune anti-HEV positive swine sera were included as negative and positive controls, respectively.

Blocking ELISA was used to confirm the results of the standard ELISA on selected anti-HEV positive and negative serum samples. The blocking ELISA for anti-HEV in swine was performed essentially as described (13) except

that the competing sera were from swine. The ORF2 protein of strain SAR55 was used for affinity purification of anti-HEV from convalescent serum of a chimpanzee exposed twice to HEV (8). The affinity-purified chimpanzee anti-HEV was conjugated with horseradish peroxidase by a custom service (ViroStat, Portland, ME), and used for the blocking ELISA (13). A serum sample was considered positive in the blocking ELISA if the OD value was reduced by ≥50% compared to the unblocked sample.

Prospective study. Twenty-one sows from a commercial herd were tested for IgG anti-HEV. Subsequently, twenty piglets (10 male and 10 female) were chosen from those born to seronegative sows (6 piglets) and to seropositive sows with a lower titer (6 piglets) or higher titer (8 piglets) of IgG anti-HEV. These 20 study piglets were tagged and mixed with other piglets from approximately 50 sows in the herd, and were commingled in two rooms in a nursery building. Piglets within a room were separated into pens by fences which allowed for nose-to-nose contacts. By the age of about 10 weeks, all piglets in the nursery building were moved to a finishing building that had been previously emptied and disinfected.

Blood samples and nasal and rectal swabs from the 20 study piglets were collected in alternate weeks from 2 weeks onward, and weekly after 14 weeks of age. The serum samples were tested for anti-HEV, and four piglets with an increasing ELISA OD value were sacrificed. Samples of 19 different tissues and organs were collected during necropsy, fixed in 10% neutral buffered formalin and processed for routine histologic examination.

Degenerate primers for reverse transcription-

polymerase chain reaction (RT-PCR). The sequences of ten human HEV strains were aligned with a GeneWorks program (IntelliGenetics, Inc., Mountain View, CA). Based on this alignment, two sets of degenerate primers were designed and synthesized to amplify two different regions of the capsid gene. The primer positions indicated below are relative to the published sequence of a Burmese HEV strain (14). Set one primers: external 3156 (forward, position 5687-5708, 5'-AAT(C)TATGCC(A)AGTACCGGGTTG-3') and 3157 (reverse, position 6395-6417, 5'-CCCTTATCCTGCTGAGCATTCTC3'), and ternal 3158 (forward, position 5972-5993, 5'-GTT(C)ATGC(T)TT(C)TGCATACATGGCT-3') and 3159 (reverse, position 6297-6319, 5'-AGCCGACGAAATC(T)AATTCTGTC-3'). Set two primers: external 3160 (forward, position 6578-6600, 5'-GCCGAGTAT(C)GACCAGTCCACTTA-3') and 3161 (reverse, position 7105-7127, 5'-AT(C)AACTCCCGAGTTTTACCCACC-3'), and internal 3162 (forward, position 6645-6667, 5'-TGGTT(G)AATGTT(A)GCGACC(T)GGCGCG-3') and 3163 (reverse, position 7063-7085, 5'-GCTCAGCGACAGTA(T)GACTGG(A)AAA-3').

RNA extraction and RT-PCR. Total RNA was extracted by TriZol reagent (GIBCO-BRL, Gaithersburg, MD) from 100 μl of serum obtained 1 or 2 weeks before seroconversion from piglets in the prospective study. Total RNA was then reverse transcribed with one of the two degenerate primers by using SuperScript II reverse transcriptase (GIBCO-BRL, Gaithersburg, MD) at **42**°C for 1 hr, and the resulting cDNA was amplified by PCR using ampliTaq Gold polymerase (Perkin Elmer, Norwalk, CT). The PCR reaction was carried out for 39 cycles of denaturation at 94°C for 1 min, annealing at 42°C for 1 min, and extension at 72°C for 2 min, followed by a nested PCR

using 10 µl of the first round PCR product with a nested set of degenerate primers.

Amplification of the entire ORFs 2 and 3 of swine HEV. After the first PCR fragment was amplified and sequenced, we designed two sets of primers, with a swine HEV-specific primer at one end and a degenerate primer at the other end (primer sequences not shown). RT-PCR with these primers was performed essentially the same as described above. The entire ORFs 2 and 3 of swine HEV were amplified in this way by walking along the genome in both directions.

Sequence analysis. The PCR fragments were cut from 1% agarose gels and purified with a Geneclean Kit (Bio101, La Jolla, CA). Both strands were sequenced with an automated DNA Sequencer. The sequences were analyzed by the GeneWorks program. Phylogenetic analyses were conducted with the aid of the PAUP software package version 3.1.1 (David L. Swofford, Illinois Natural History Survey, Champaign, IL).

EXAMPLES

Standardization of an ELISA for swine anti-HEV. To establish a reliable serological test for anti-HEV in swine, we first generated hyperimmune antisera by immunizing two SPF pigs with recombinant HEV ORF2 antigen. IgG anti-HEV was first detected at 2 and 3 weeks postimmunization, and reached peak ELISA titers of 10^{-4} at weeks 3 and 4 postimmunization, respectively. The anti-HEV titer remained at 10^{-4} until the end of the 9 week experiment. An ELISA for swine anti-HEV was subsequently standardized by using the preimmune and hyperimmune swine

sera along with 34 normal sera from swine raised in laboratory environments and from swine in an SPF herd. The ELISA cutoff value was set at 99% confidence bounds, based on the frequency distribution of the absorbance values of normal sera. The cutoff value was approximately 2.5 standard deviations above the mean absorbance value of the normal sera.

Serologic evidence in swine for infection with an agent related to HEV. The prevalence of anti-HEV in commercial swine herds in the midwestern United States was assessed by the standardized ELISA. Surprisingly, IgG anti-HEV was found in a majority of swine ≥3 months of age in herds in the midwestern United States where human hepatitis E is not endemic; however, most swine ≤2 months of age were seronegative (Table 1). None of the 10 adult swine from an SPF herd was seropositive (Table 1). To further validate the serology results, we performed a blocking ELISA on selected anti-HEV positive and negative swine sera. Similar results were obtained in the blocking ELISA and the standard ELISA. The HEV antigen used in ELISA was expressed in insect cells; therefore we also included insect cells infected with baculovirus lacking the HEV sequence as a negative antigen control and HPLC-purified HEV recombinant antigen as a positive antigen control. There was little or no reaction between the swine serum samples and the insect cells infected with the baculovirus lacking the HEV sequence. Thus, the swine anti-HEV reacted specifically with the human HEV capsid antigen in the standard ELISA and completed with anti-HEV in convalescent chimpanzee serum in the blocking ELISA. These data strongly suggested that a ubiquitous swine agent, antigenically related to human HEV, was circulating

in the general swine population.

Natural infection of swine in a commercial herd. In an attempt to identify this putative HEV-related agent in pigs, a prospective study was conducted in a commercial swine herd in the midwestern United States. Consistent with our seroepidemiological results, 18 of 21 pregnant sows tested from this herd were positive for anti-HEV. Piglets born to seronegative sows were seronegative at 2 weeks of age, and piglets born to seropositive sows with a lower titer of IgG anti-HEV also scored as seronegative but had a comparatively high ELISA OD value for IgG anti-HEV as shown in Table 2.

Table 2. Seroconversion of piglets to anti-HEV in a commercial herd: a prospective study

Piglet No.	Breeder sow ELISA OD*	Piglet ELISA OD*		Age seroconverted (wks)
		2 wks	8 or 9 wks	
15	0.908	0.550	0.036	N†
16	0.908	0.522	0.039	19
17	0.908	0.501	0.066	Neg‡
18	1.011	0.822	0.103	N†
19	1.011	1.264	0.148	15
20	1.011	0.979	0.146	Death§
1	0.692	0.128	0.024	18
2	0.692	0.211	0.071	21
3	0.692	0.157	0.052	20
4	0.692	0.114	0.026	18
5	0.431	0.107	0.065	18
6	0.431	0.216	0.059	21
7	0.424	0.113	0.073	16N†
8	0.424	0.195	0.093	15
9	0.209	0.047	0.050	18
10	0.209	0.079	0.090	19
11	0.209	0.047	0.039	19
12	0.245	0.057	0.061	14

Table 2. Seroconversion of piglets to anti-HEV in a commercial herd: a prospective study

Piglet No.	Breeder sow ELISA OD*	Piglet ELISA OD*		Age seroconverted (wks)
		2 wks	8 or 9 wks	
13	0.245	0.056	0.038	16
14	0.245	0.057	0.012	20N†

*Elisa cut-off value: 0.3; N†: necropsied

‡Neg: remains seronegative at 21 weeks of age

§Death due to unknown cause

In contrast, piglets born to seropositive sows with a higher titer of IgG anti-HEV were positive at 2 weeks of age for IgG anti-HEV (Table 2), but not for IgM anti-HEV. The level of IgG anti-HEV in seropositive piglets decreased dramatically within a few weeks after birth and had disappeared by the age of 8 or 9 weeks (Table 2, Fig. 1). Clearly, the anti-HEV detected in these newborns represented maternal antibody as evidenced by the correlation between the levels of anti-HEV in 2 weeks old piglets and in their dams (Table 2), and from the fact that the anti-HEV belonged to the IgG class. However, after the maternal antibody had waned, most of the piglets had developed their own antibodies to HEV. One piglet seroconverted to anti-HEV at the age of 14 weeks, followed within a few weeks by seroconversion of piglets in other pens housed in the same finishing building. The pattern of anti-HEV appearance, starting with piglets grouped near the first seropositive piglet, then followed by more distal ones, was consistent with seroconversion induced by an infectious agent (data not shown). By 21 weeks of age, 17 of the 20 piglets had seroconverted. Two other piglets were necropsied prior to seroconversion and one piglet died of an unknown cause. The only remaining

seronegative piglet had a rising ELISA OD value but it was still below the cut-off value at the end of the 21 week study. The level of IgG anti-HEV increased steadily for several weeks after seroconversion (Fig. 1). IgM anti-HEV, indicating a newly contracted infection with this putative HEV-related agent, was also detected in all piglets which seroconverted to IgG anti-HEV. The level of IgM anti-HEV peaked about one week earlier than that of IgG anti-HEV and decreased rapidly over about 1 to 2 weeks (Fig. 1).

Clinical illness was not observed in the piglets. Four piglets believed to be at an early stage of infection were necropsied during the study. Except for a gross lung lesion consistent with a bacterial pneumonia in one piglet, other gross lesions were not apparent in 19 different tissues and organs examined during necropsy. Microscopically, all 4 piglets necropsied had evidence of hepatitis characterized by mild to moderate multifocal and periportal lymphoplasmacytic hepatitis with mild focal hepatocellular necrosis. In addition, all piglets had lymphoplasmacytic enteritis, and three piglets also had mild multifocal lymphoplasmacytic interstitial nephritis. Syncytial cells were also noticed in the tonsils and Peyer's patches of one piglet (data not shown).

Genetic characterization of the swine HEV. Since the swine anti-HEV reacted so strongly with the capsid protein of human HEV, it was probable that swine HEV shared nucleotide sequence similarity with human HEV. Therefore, two sets of degenerate primers derived from the HEV putative capsid gene were used to attempt the amplification of the swine HEV genome by RT-PCR of serum samples obtained 1 and 2 weeks before seroconversion. A

fragment representing part of the swine HEV genome (Fig. 2) was first amplified by a nested-PCR with primer set 3158 and 3159. Sequence information confirmed that this initial PCR fragment was specific for swine HEV and represented part of the ORF2.

Sequence analyses of swine HEV ORFs 2 and 3. Analyses of the complete ORF 2 and 3 sequences revealed that swine HEV is closely related to, although distinct from, human HEV strains. In the putative capsid gene (ORF2), swine HEV shares with human HEV strains about 79 to 80% sequence identify at the nucleotide level, and about 90 to 92% identity at the amino acid level (Table 3, Fig. 3).

Table 3. Pairwise comparison of the nucleotide and deduced amino acid sequences of the open reading frames (ORFs) 2 and 3 of the swine hepatitis E virus (SHEV) with human HEV strains

Virus Strains	SHEV	Mexico	HEV-037	Uigh-179	Hetian	KS2-87	Sar55	Madras	Hyde-rabad	Burma	NE8L
					ORF2						
SHEV		79(90)	80(91)	80(91)	80(91)	80(92)	80(92)	79(92)	79(90)	79(92)	79(91)
Mexico	83(79)*		81(93)	81(92)	81(93)	81(93)	81(93)	81(93)	81(92)	81(93)	81(92)
HEV-037	84(82)	90(89)		94(98)	94(98)	94(98)	94(99)	94(98)	92(97)	94(98)	94(98)
Uigh-179	84(80)	90(85)	97(97)		98(98)	98(99)	97(99)	93(98)	93(97)	94(99)	93(98)
Hetian	84(80)	90(85)	97(97)	98(97)		99(99)	98(99)	93(98)	93(97)	94(99)	93(98)
KS2-87	84(80)	90(85)	97(97)	98(98)	98(97)		98(99)	93(99)	93(98)	94(99)	94(98)
Sar55	85(82)	91(87)	98(98)	99(98)	99(98)	99(98)		93(99)	93(98)	94(99)	93(99)
Madras	85(82)	90(87)	98(98)	98(98)	98(98)	98(98)	99(100)		96(98)	97(99)	96(98)
Hyderabad	83(77)	89(84)	95(93)	96(93)	96(93)	97(95)	97(95)	97(95)		97(98)	96(97)
Burma	84(82)	90(87)	98(98)	98(98)	98(98)	98(98)	99(100)	99(100)	97(95)		98(99)
NE8L	84(81)	90(87)	98(97)	98(97)	97(97)	97(97)	98(98)	98(98)	96(93)	99(98)	
					ORF3						

*The values in the table represent the percentage of nucleotide or amino acid (in bracket) sequences

However, the relatively high amino acid identity between swine and human HEV is significantly lower than

the amino acid identity (97 to 99%) among human HEV strains with the exception of the Mexican strain. The Mexican strain of HEV also displayed greater sequence divergence of about 92 to 93% amino acid identity with other human HEV strains (Table 3). However, the genetic distances between swine HEV and the Mexican strain of HEV are comparable to those between swine HEV and other human HEV strains, indicating that swine HEV is also distinct from the Mexican HEV (Table 3, Fig. 3). These data suggested that we had identified a previously unrecognized swine virus belonging to the same family as human HEV.

The small ORF3 of swine HEV had about 83 to 85% sequence identity at the nucleotide level with human HEV strains, but only 77 to 82% identity at the amino acid level (Table 3). The human HEV strains also displayed a lower percentage of identities at the amino acid level than at the nucleotide level (Table 3). Most of the amino acid variations in ORF3 were clustered in a hypervariable region consisting of 17 amino acid residues near the carboxyl terminus (Fig. 3). In addition, the ORF3 of swine HEV had a single amino acid deletion near the amino-terminus (Fig. 3).

The evolutionary relationships between swine and human HEV were determined on the basis of the complete nucleotide sequences of ORF2 and ORF3. The resulting phylogenetic tree revealed that human HEV strains were represented by at least two genotypes. The first genotype was represented by the Mexican strain, and the second genotype by the other human HEV strains (Fig. 4). Phylogenetically, swine HEV was unique, the most divergent of the HEV strains compared and the first member of a third genotype (Fig. 4).

Experimental infection of SPF swine with swine HEV. SPF swine were inoculated intravenously with acute phase serum samples from the prospective study already described. The inoculated swine were monitored for anti-HEV response, viremia, and liver enzymes. The inoculated swine seroconverted to anti-HEV at 5 weeks post-inoculation, and viremia appeared one week before the seroconversion (Fig. 5). The incubation period for swine HEV is about 4 to 5 weeks since the control (uninoculated) pig housed in the same room seroconverted 8 weeks post-inoculation. A pig infected apparently by contact also developed viremia as measured by RT-PCR with swine HEV-specific primers. Viremia occurred 2 weeks before seroconversion (i.e., 6 weeks post-inoculation) and lasted for 3 weeks.

Amplification of the complete genome of swine HEV. In order to extend the sequence of ORFs 2 and 3 of swine HEV to ORF1, a genome-walking strategy was utilized (Fig. 8). The complete genome of swine HEV was amplified by RT-PCR by using one swine HEV-specific primer and one HEV degenerate or consensus primer (Fig. 8). The PCR reaction conditions used to amplify different regions of the genome varied.

Sequence analysis of ORF1 and terminal NC regions of swine HEV. The nucleotide and deduced amino acid sequences of ORF1 are shown in Figures 6A-6J and the complete genomic sequence is shown in Figure 7A-7D.

The putative functional domains and the hypervariable region (HVR) in the ORF1 were compared with the corresponding regions of other HEV strains. The ORF1 of swine HEV and of the US-2 strain contain 5127

nucleotides (nts), which is 3 nts less than in the US-1 strain, but 45 and 51 nts more than in the Asian strains and the Mexico strain, respectively (data not shown). Swine HEV varied extensively, both at the nucleotide and amino acid levels, from non-U.S. strains of HEV although it was very similar to the two US strains. The sequence identity in the putative methyltransferase and RDRP regions between swine HEV and non-U.S. HEV strains varied from 74 to 76% at the nucleotide level, and 84 to 89% at the amino acid level. The GDD tripeptide motif found in all viral RDRP is conserved among different strains (data not shown). In the putative helicase region, a slightly higher sequence identity between swine HEV and non-U.S. HEV strains was observed, 74 to 77% at the nucleotide level and 91 to 92% at the amino acid level. Asian strains of HEV, Burma (14), Myanmar (16), Pakistan (18), China (19) and Madras (India, Genbank accession no. X99441) are closely related to each other. The ORF1 of the Mexican strain of HEV (15), like ORFs 2 and 3, also showed much greater sequence divergence from other HEV strains, ranging from 73 to 80% sequence identity at the nucleotide level and 85 to 94% at the amino acid level. However, the sequence identity between swine HEV and the Mexican strain was as divergent as those between swine HEV or the Mexican strain and non-U.S. strains of HEV.

The 3′ NCR of swine HEV was also amplified and sequenced. The primer sequence in the extreme 3′-end was excluded, and the 3′ NCR region containing the remaining 54 bp of swine HEV was compared with the corresponding regions of other HEV strains. The 3′ NCR of swine HEV appeared to be very divergent: it shared about 87% sequence identity with that of the US-1 strain, but only

about 58 to 70% sequence identity with the corresponding regions of the Asian strains. The 3′ NCR of the Mexican strain is the longest, and varied extensively from all other HEV strains, including US-1 and swine HEV. In contrast, the 3′ NCRs among the Asian strains were very conserved, ranging from 96 to 98% nucleotide sequence identity. The 5′ NCR of swine HEV was also amplified. However, only 9 nucleotides were left in the 5′ NCR of swine HEV after excluding the primer sequence used for the amplification. Therefore, further analysis of this region was not performed.

Experimental inoculation of non-human primates with swine HEV.

Two rhesus monkeys, RH-H397 (female) and RH-H398 (male) and one female chimpanzee (CH-5835) were each inoculated (Week 0) intravenously with 0.5 ml (rhesus) or 1.0 ml (chimpanzee) of a 10% fecal suspension (equivalent to approximately 10^3 rhesus infectious doses) of swine HEV. Weekly serum samples were tested for anti-HEV by ELISA and for ALT levels by standard methods. The results are shown in Table 4.

Table 4

	ALT (U/L)			Anti-HEV IgG		
Week Post-inoculation	RH-H397	RH-H398	CH-5835	RH-H397	RH-H398	CH-5835
0	42	35	29	< 1:100[1]	< 1:100	<1:100
1	43	30	26	< 1:100	< 1:100	< 1:100
2	43	33	27	< 1:100	< 1:100	< 1:100
3	97	38	31	< 1:100	< 1:100	< 1:100
4	81	39	30	1:100	1:100	< 1:100
5	96	37	26	1:100	1:1000	< 1:100
6	67	32	35	1:100	1:100	1:100
7	54	47	28	1:10,000	1:10,000	1:100
8	44	35	25	1:10,000	1:10,000	1:1,000
9	50	37	30	1:10,000	1:1,000	1:100
10	44	55	23	1:1,000	1:1,000	1:100
11	49	37	33	1:1,000	1:100	1:100
12	59	41	25	1:1,000	1:1,000	1:100
13	40	30	34	1:1,000	1:100	1:100
14	52	34	[2]ND	1:1,000	1:1,000	ND

[1] The dilution presented is the dilution of sera which gave a positive response for anti-HEV IgG

in ELISA. A positive response with a dilution of 1:100 or greater is considered evidence of serconversion.

[2] ND = not determined.

Animals RH-H398 and CH-5835 showed no elevation in ALT levels following inoculation with the swine HEV while RH-H397 showed a slight increase in ALT levels at weeks 3, 4 and 5 post-inoculation.

In addition, both rhesus monkeys seroconverted at week 4 postinoculation while the chimpanzee seroconverted at week 6 postinoculation. Thus, the data presented in Table 4 demonstrates that in surrogates of man, the swine HEV of the present invention is completely or almost completely attenuated and elicits a strong antibody response.

Cross Challenge of Rhesus Monkeys Previously Infected With Swine HEV

Rhesus monkey RH-H397 is to be challenged intravenously with 0.5 ml (a 10^{-2} dilution of the SAR55 stool pool diluted an additional 1:3 with PBS before inoculation) of 10^4 monkey infectious doses (MID_{50}) of the SAR55 Pakistani strain of HEV (18) and Rhesus monkey RH-H398 is to be challenged intravenously with 0.5 ml (a 10^{-2} dilution of the MEX14 stool pool) of 10^4 MID_{50} of the MEX-14 Mexican strain of HEV (15) respectively.

Post-challenge, weekly serum samples are obtained and tested for viral RNA, anti-HEV and ALT levels by standard methods.

REFERENCES

1. Purcell, R.H. (1996) in *Fields Virology*, eds. Fields, B.N., Knipe, D.M., Howley, P.M. et al. (Lippincott-Raven Publishers, Philadelphia), 3rd ed. Vol. 2, pp. 2831-2843.
2. Wong, D.C., Purcell, R.H., Sreenivasan, M.A., Prasad, S.R. & Pavri, K.M. (1980) *Lancet***2**, 876-878.
3. Arankalle, V.A., Tsarev, S.A., Chadha, M.S., Alling, D.W., Emerson, S.U., Banerjee, K. & Purcell, R.H. (1995) *J. Infect Dis* **171** 447-450.
4. Bradley, D.W. (1992) *Rev Med Virol* **2**, 19-28.
5. Skidmore, S.J., Yarbough, P.O., Gabor, K.A., Tam, A.W. & Reyes, G.R. (1991) *Lancet***337**, 1541.
6. Dawson, G.J., Mushahwar, I.K., Chau, K.H. & Gitnick, G.L. (1992) *Lancet***340**, 426-427.
7. Dawson, G.J., Chau, K.H., Cabal, C.M., Yarbough, P.O., Reyes, G.R. & Mushahwar, I.K. (1992) *J Virol Methods* **38**, 175-186
8. Tsarev, S.A., Tsareva, T.S., Emerson, S.U., Kapikian, A.Z., Ticehurst, J., London, W. & Purcell, R.H. (1993) *J. Infect. Dis.* **168**, 369-378.
9. Thomas, D.L., Yarbough, P.O., Vlahov, D., Tsarev, S.A., Nelson, K.E., Saah, A.J. & Purcell, r.H. (1997) *J. Clin. Microbiol*. **35**, 1244-1247.
10. Clayson, E.T., Innis, B.L., Myint, K.S.A., Narupiti, S., Vaughn, D.W., Giri, S., Ranabhat, P. & Shrestha, M.P. (1995) *Am J Trop Med Hyd* **53**, 228-232.
11. Balayan, M.S., Usmanov, R.K., Zamyatina, D.I. & Karas, F.R. (1990) *J Med Virol* **32**, 58-59.

12. Tsarev, S.A., Emerson, S.U., Tsareva, T.S., Yarbough, P.O., Lewis, M., Govindarajan, S., Reyes, G.R., Shapiro, M. & Purcell, R.H. (1993) *J. Infect. Dis.* **167**, 1302-1306.

13. Tsarev, S.A., Tsareva, T.S., Emerson, S.U., Rippy, M.K., Zack, P., Shapiro, M. & Purcell, R.H. (1995) *J. Infect. Dis.* **172**, 31-37.

14. Tam, A.W., Smith, M.M., Guerra, M.E., Huang, C.-C., Bradley, D.W., Fry, K.E. & Reyes, G.R. (1991) *Virology* **185**, 120-131.

15. Huang, C.C., Nguyen, D., Fernandez, J., Yun, K.Y., Fry, K.E., Bradley, D.W., Tam, A.W. & Reyes, G.R. (1992) *Virology* **191**, 550-558.

16. Aye, T.T. Uchida, T., Ma, X.-Z., Iida, F. Shikata, T., Ichikawa, M., Rikihisa, T. & Win, K.M (1993) *Virus Genes* **7**, 95-110.

17. Panda, S.K., Nanda, S.K., Zafrullah, M., Ansari, I.H., Ozdener, M.H. & Jameel, S. (1995) *J. Clin. Microbiol.* **33**, 2653-2659.

18. Tsarev, S.A., Emerson, S.U., Reyes, G.R., Tsareva, T.S., Legters, L.J., Malik, I.A., Iqbal, M. & Purcell, R.H. (1992) *Proc. Natl. Acad. Sci. USA* **89**, 559-563.

19. Yin, S., Purcell, H.H. & Emerson, S.U. (1994) *Virus Genes* **9**, 23-32.

20. Aye, T.T., Uchida, T., Ma, X.-Z., Iida, F., Shikata, T., Zhuang, H. & Win, K.M. (1992) *Nucleic Acids Res.* **20**, 3512.

21. Hollinger, F.B. & Ticehurst, J. (1990) in *Fields Virology* eds. Fields, B.N., Knipe, d.M. Et al. (Raven Press, Ltd., New York), 2nd ed. Vol. 1, pp. 631-667.

22. Grieder, F.B., Davis, N.L., Aronson, J.F., Charles, P.C., Sellon, D.C., Suzuki, K. & Johnston, R.E. (1995) *Virology* **206**, 994-1006.

23. Park, B.H., Matuschke, B., Lavi, E. & Gaulton, G.N. (1994) *J. Virol.* **68**, 7516-7524.

24. Murphy, F.A. (1996) *Science* **273**, 746-747.

CLAIMS

1. A swine hepatitis E virus, or natural mutants thereof, capable of cross-reacting with antibodies reactive with a human HEV virus strain or natural mutants thereof.

2. The swine hepatitis E virus of claim 1 wherein the nucleic acid sequence of the genome of said virus shares homology with the nucleic acid sequence of the genome of human hepatitis E virus or naturally occurring mutants thereof.

3. The swine hepatitis E virus of claim 1 wherein the nucleic acid sequence of the genome of said virus contains 3 open reading frames, designated ORF1, ORF2 and ORF3.

4. The swine hepatitis E virus of claim 3 wherein the ORF2 nucleic acid sequence encodes a capsid protein.

5. The swine hepatitis E virus of claim 3 wherein the ORF2 nucleic acid sequence possesses at least about 79% nucleotide sequence identity with the corresponding ORF2 sequence of a human hepatitis E virus or naturally occurring mutants thereof.

6. The swine hepatitis E virus of claim 3 wherein the ORF2 nucleic acid sequence encodes a protein having at least about 90% amino acid sequence identity with a capsid protein of human hepatitis E virus.

7. The swine hepatitis E virus of claim 3 wherein the ORF3 nucleic acid sequence possesses at least about 83% nucleotide sequence identity with the corresponding ORF3 sequence of a human hepatitis E virus or naturally occurring mutants thereof.

8. The swine hepatitis E virus of claim 3 wherein the ORF3 nucleic acid sequence encodes a protein having at least about 77% amino acid sequence identity with the ORF3 protein of a human hepatitis E virus.

9. The ORF1 nucleic acid sequence of the swine hepatitis E virus of claim 1.

10. The ORF2 nucleic acid sequence of the swine hepatitis E virus of claim 1.

11. The ORF3 nucleic acid sequence of ORF3 of the swine hepatitis E virus of claim 1.

12. A recombinant protein encoded by the nucleic acid sequence of claims 9, 10 or 11.

13. The protein of claim 12 wherein said protein is highly purified.

14. The protein of claim 12 wherein said protein is the capsid protein of HEV encoded by ORF2.

15. The protein of claim 12 wherein said protein is the protein encoded by ORF3.

16. The protein of claim 12, wherein said protein is the protein encoded by ORF1.

17. A method of determining the susceptibility of cells *in vitro* to support swine HEV infection, comprising the steps of:

a. growing animal cells in vitro;

b. infecting said cells with the virus of claim 1; and

c. determining if said cells show indicia of HEV replication.

18. The method according to claim 17 wherein said cells are human cells.

19. An antibody specific for a protein encoded by the nucleic acid sequence according to claims 9, 10 or 11.

20. The antibody of claim 19 wherein said antibody is a polyclonal antibody.

21. The antibody of claim 19, wherein said antibody is a monoclonal antibody.

22. The antibody of claim 19 wherein said antibody is labeled with a detectable label.

23. A method for detecting antibody to a swine HEV in a biological sample, said method comprising the steps of:

a. contacting the sample with a protein according to claims 9, 10 or 11 under conditions suitable to form a complex with said antibodies; and

b. detecting the presence of said complex in said sample.

24. A diagnostic test kit for detection of HEV antigens in a test sample, said kit comprising in combination:

a. a container containing a specific amount of HEV specific antibody;

b. a container containing an optional diluent for suspension of said test sample;

c. a container containing a sample of a positive control for said assay; and

d. a container containing a sample of a negative control for said assay.

25. The diagnostic test kit according to claim 24 wherein the antibody is the antibody of claim 19.

26. A vaccine for use in immunizing against HEV, comprising a pharmaceutically active composition containing a protein encoded by the nucleic acid sequence according to claims 9, 10 or 11 and suspended in a pharmaceutically acceptable diluent or excipient.

27. A composition comprising a protein of claim 12 suspended in a suitable amount of a pharmaceutically acceptable diluent or excipient.

28. A method for treating hepatitis E viral infection comprising the administration to a mammal in need thereof of a clinically effective amount of the composition of claim 26.

29. The method of claim 28 wherein said mammal is a human.

30. A vaccine for use in immunizing against HEV, comprising a pharmaceutically active composition containing swine hepatitis E virus according to claim 1 and suspended in a pharmaceutically acceptable diluent or excipient.

31. The vaccine of claim 30 wherein the swine hepatitis E virus is a live virus.

32. The vaccine of claim 30 wherein the swine hepatitis E virus is a killed virus.

33. A method for assessing the risk of swine HEV infection in xenotransplantation of pig organs to other animals, comprising the steps of:

a. obtaining a biological sample of a tissue from a pig to be used as a source of organs for transplant (the donor pig);

b. testing the biological sample for the presence of swine HEV.

34. The method of claim 33 wherein the testing is done by detecting the formation of a complex between the antibodies of claim 19 and the biological sample.

35. The method of claim 33 wherein the testing is done by the procedure comprising:

a. extracting RNA from the biological sample;

b. using probes complementary to unique swine HEV RNA sequences, with optional RT-PCR to amplify such sequences if necessary; and

c. detecting the binding of said probes to said sequences, thereby indicating the presence of swine HEV in said sample.

36. A swine hepatitis E virus whose ORF2 protein possesses at least about 93% amino acid sequence identity with the ORF2 amino acid sequence shown in Figure 3A.

37. The swine hepatitis E virus of claim 36, wherein the ORF2 protein of said virus possesses at least about 95% amino acid sequence identity with the ORF2 amino acid sequence shown in Figure 3A.

38. The swine hepatitis E virus of claim 37, wherein the ORF2 protein of said virus possesses at least about 97% amino acid sequence identity with the ORF2 amino acid sequence shown in Figure 3A.

39. A swine hepatitis E virus whose ORF3 protein possesses at least 85% amino acid sequence identity with the ORF3 amino acid sequence shown in Figure 3B.

40. The swine hepatitis E virus of claim 39 wherein the ORF3 protein of said virus possesses at least about 90% amino acid sequence identity with the ORF3 amino acid sequence shown in Figure 3B.

41. The swine HEV of claim 40, wherein the ORF3 protein of said virus possesses at least about 95% amino acid sequence identity with the ORF3 amino acid sequence shown in Figure 3B.

42. A swine HEV whose ORF1 protein possesses at least about 85% amino acid sequence identity, with the ORF1 amino acid sequence shown in Figures 6D-6J.

43. The swine HEV of claim 42, wherein the ORF1 protein of said virus possesses at least about 90% amino acid sequence identity with the ORF1 amino acid sequence shown in Figures 6D-6J.

44. The swine HEV of claim 43, sherein the ORF1 protein of said virus possesses at least about 95% amino acid sequence identity with the ORF1 amino acid sequence shown in Figures 6D-6J.

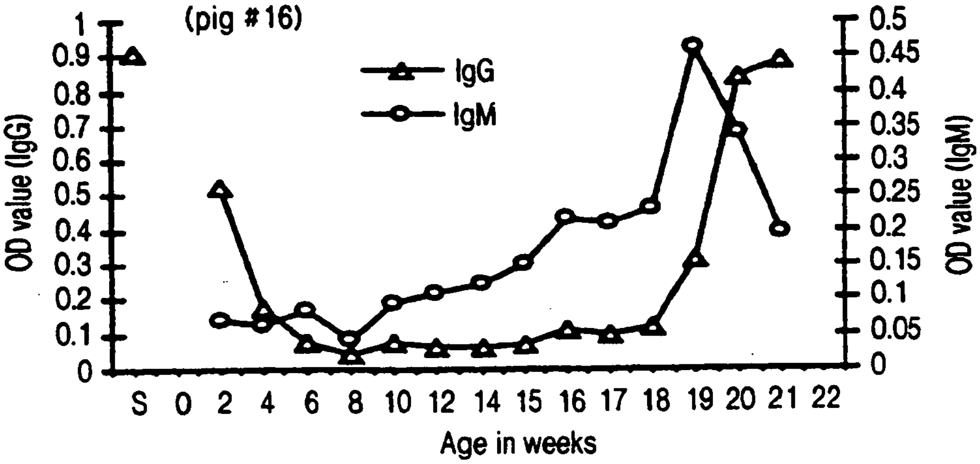


FIG. 1A

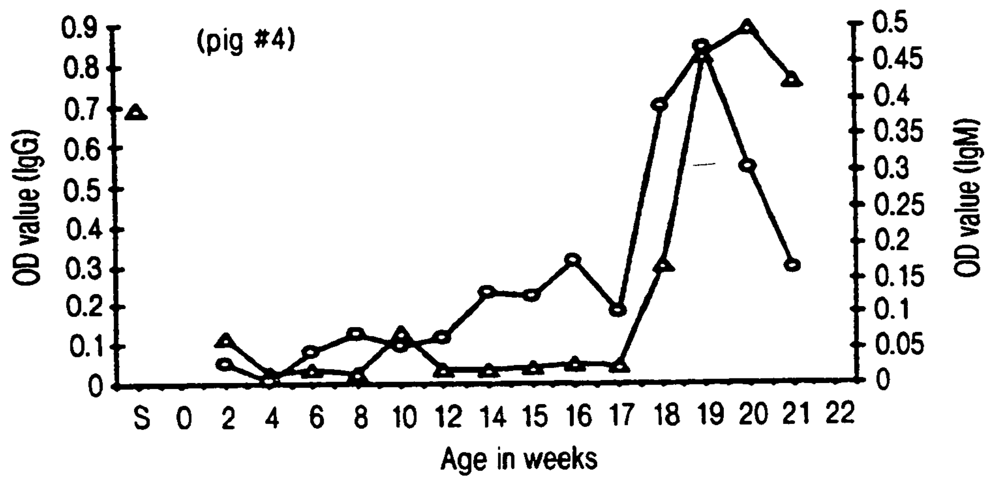


FIG. 1B

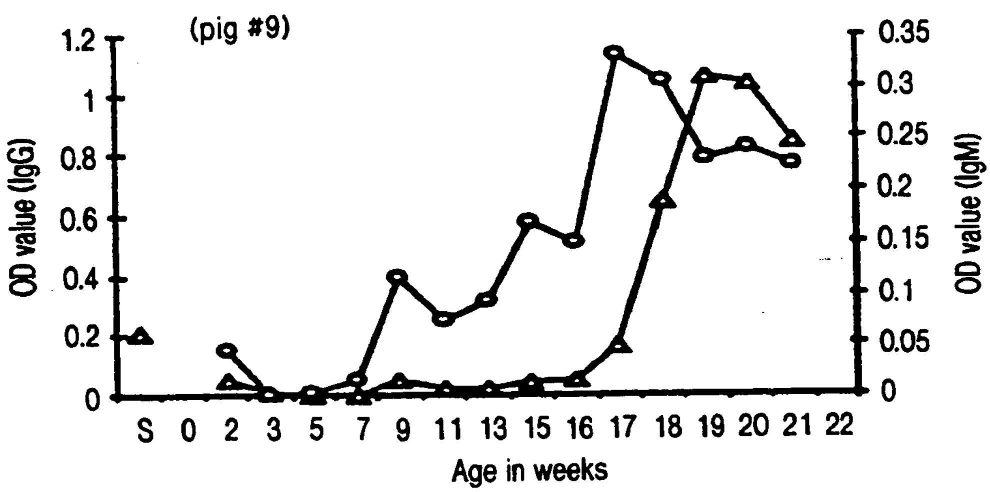


FIG. 1C

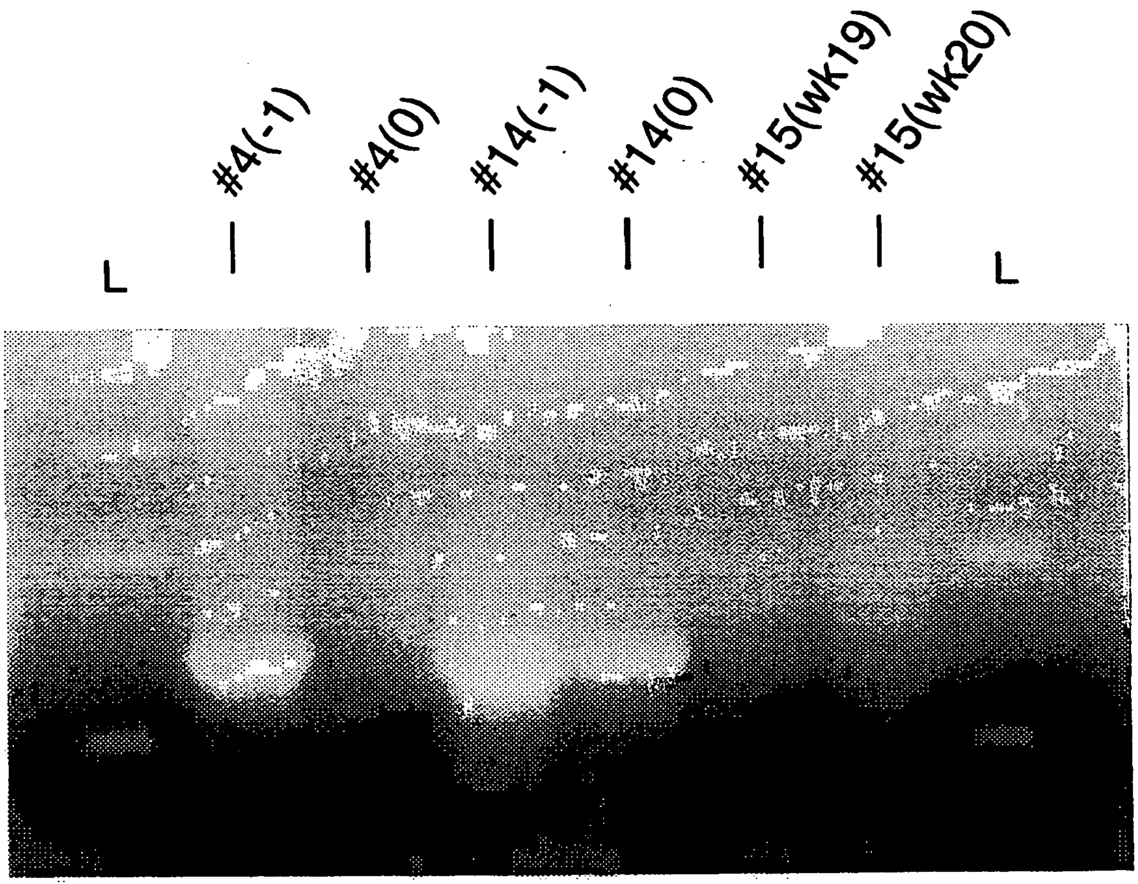


FIG. 2

```
Sar55      MRPRPILLLLLMFLPMLPAPPPGQPSGRRRGRRSGGSGGGFWGDRVDSQPFAIPYIHPTN 60
Mexico     .....L...F.L.........T..............T....................... 60
KS2-87     ............................................................ 60
Burma      ............................................................ 60
Madras     ............................................................ 60
Uigh179    ...............IV........................................... 60
HEV037     ......................................................H..... 60
Hyderabad  .G.......F.......L.......................................... 60
NE8L       ............................................................ 60
Hetian     .............................................A.............. 60
Swine HEV  ....AV....FVL........A.......C...N..A...............L....... 60

Sar55      PFAPDVTAAAGAGPRVRQPARPLGSAWRDQAQRPAAASRRRPTTAGAAPLTAVAPAHDTP 120
Mexico     ......A..S.S...L.........T........S.............A..........S 120
KS2-87     ...................V.......................A................ 120
Burma      ...................................V........................ 120
Madras     ..................S..................P...................... 120
Uigh179    ............................................................ 120
HEV037     ....................................T....................... 120
Hyderabad  ....N..............V........................................ 120
NE8L       ...................................V........................ 120
Hetian     ............................................................ 120
Swine HEV  ...A..VSQP...D.P...P..........S...ST.P...SAP.........S..P..A 120

Sar55      PVPDVDSRGAILRRQYNLSTSPLTSSVATGTNLVLYAAPLSPLLPLQDGTNTHIMATEAS 180
Mexico     ............................S...........N.P................. 180
KS2-87     ............................................................ 180
Burma      ............................................................ 180
Madras     ............................................................ 180
Uigh179    ............................................................ 180
HEV037     .........................P.................................. 180
Hyderabad  ............................................................ 180
NE8L       ........A................................................... 180
Hetian     ............................................................ 180
Swine HEV  ............................A...........N................... 180

Sar55      NYAQYRVARATIRYRPLVPNAVGGYAISISFWPQTTTTPTSVDMNSITSTDVRILVQPGI 240
Mexico     ............................................................ 240
KS2-87     ............................................................ 240
Burma      ............................................................ 240
Madras     ............................................................ 240
Uigh179    .........................G.................................. 240
HEV037     ............................................................ 240
Hyderabad  ....................................P....................... 240
NE8L       .......V.................................................... 240
Hetian     .......V.................................................... 240
Swine HEV  .......V.................................................... 240
```

FIG. 3A-1

```
Sar55      ASELVIPSERLHYRNQGWRSVETSGVAEEEATSGLVMLCIHGSPVNSYTNTPYTGALGLL 300
Mexico     ............................................................ 300
KS2-87     ............................................................ 300
Burma      ...........................................L................ 300
Madras     ............................................................ 300
Uigh179    ..........................................................V. 300
HEV037     ..........................................L................. 300
Hyderabad  ............................................................ 300
NE8L       ............................................................ 300
Hetian     ...H.......................................L................ 300
Swine HEV  .......................T.................................... 300

Sar55      DFALELEFRNLTPGNTNTRVSRYSSTARHRLRRGADGTAELTTTAATRFMKDLYFTSTNG 360
Mexico     ............TC...............SA-.....................H..GL.. 360
KS2-87     ............................................................ 360
Burma      ............................................................ 360
Madras     ............................................................ 360
Uigh179    ..................................T......................... 360
HEV037     .....F...................................................... 360
Hyderabad  ............................................................ 360
NE8L       ............................................................ 360
Hetian     ............................................................ 360
Swine HEV  .......................T.............................H..G... 360

Sar55      VGEIGRGIALTLFNLADTLLGGLPTELISSAGGQLFYSRPVVSANGEPTVKLYTSVENAQ 420
Mexico     ...V........L............................................... 420
KS2-87     ............................................................ 420
Burma      ............................................................ 420
Madras     .....................................................R...... 420
Uigh179    ............................................................ 420
HEV037     ............................................................ 420
Hyderabad  ............................................................ 420
NE8L       ............................................H............... 420
Hetian     ............................................................ 420
Swine HEV  ...V........................................................ 420

Sar55      QDKGIAIPHDIDLGESRVVIQDYDNQHEQDRPTPSPAPSRPFSVLRANDVLWLSLTAAEY 480
Mexico     ....V.........D............................................. 480
KS2-87     ............................................................ 480
Burma      ............................................................ 480
Madras     .................................................A.......... 480
Uigh179    ............................................................ 480
HEV037     ............................................................ 480
Hyderabad  ........N................................................... 480
NE8L       ............................................................ 480
Hetian     ............................................................ 480
Swine HEV  .....T........D............................................. 480
```

FIG. 3A-2

```
Sar55       DQSTYGSSTGPVYVSDSVTLVNVATGAQAVARSLDWTKVTLDGRPLSTIQQYSKTFFVLP 540
Mexico      .............I......................S.........P.VE.......... 540
KS2-87      ............................................................ 540
Burma       ............................................................ 540
Madras      ............................................................ 540
Uigh179     ....................................................C....... 540
HEV037      ............................................................ 540
Hyderabad   ......................................................I..... 540
NE8L        .........A..........................................P....... 540
Hetian      ................................................T........... 540
Swine HEV   ..T......N.M....T...................S.........T.........Y... 540

Sar55       LRGKLSFWEAGTTKAGYPYNYNTTASDQLLVENAAGHRVAISTYTTSLGAGPVSISAVAV 600
Mexico      ............................I.I...............R......A...A.. 600
KS2-87      ............................................................ 600
Burma       ............................................................ 600
Madras      ............................................................ 600
Uigh179     ............................................................ 600
HEV037      ..............................I......................A...... 600
Hyderabad   .............RP............................................. 600
NE8L        ............................................................ 600
Hetian      ............................................................ 600
Swine HEV   ............................I.I.....................T.....G. 600

Sar55       LAPHSVLALLEDTMDYPARAHTFDDFCPECRPLGLQGCAFQSTVAELQRLKMKVGKTREL 660
Mexico      ...R.A.......F...G.............A...................V........ 660
KS2-87      .T...A...................................................... 660
Burma       .....A.......L.............................................. 660
Madras      .....A.......L.............................................. 660
Uigh179     .....A...................................................... 660
HEV037      .....A...................................................... 660
Hyderabad   .G...A.......L.............................................. 660
NE8L        .....A.......L...C.......................................... 660
Hetian      .....A...................................................... 660
Swine HEV   .....A..V....V.................T...........I...............S 660
```

FIG. 3A-3

```
Sar55       MNNMSFAAPMGSRPCALGLFCCCSSCFCLCCPRHRPVSRLAAVVGGAAAVPAVVSGVTGL 123
Mexico      ....W.......P............................................... 123
Madras      ............................................................ 123
Burma       ............................................................ 123
KS2-87      ...........P................................................ 123
NE8L        ...............D............................................ 123
HEV037      ............................................................ 123
Uigh179     ...........W.................S.............................. 123
Hetian      .....S....................................A................. 123
Hyderabad   .D............W................S............................ 123
Swine HEV   .......S....-.......................A...........T........... 123

                                ****************** (HVR, position 79-96)
Sar55       ILSPSQSPIFIQPTPSPPMSPLRPGLDLVFANPPDHSAPLGVTRPSAPPLPHVVDLPQLGPRR 123
Mexico      ...............L.QTL........A...Q.G.L....EI........P.A....P.L.. 123
Madras      ............................................................... 123
Burma       ............................................................... 123
KS2-87      .................................S............................. 123
NE8L        ................................Q.............................. 123
HEV037      ...............L............A.................................. 123
Uigh179     ............................................................... 123
Hetian      ............................................................... 123
Hyderabad   .................R...............S.......A..................... 123
Swine HEV   .....P..........L...FHN...EFALDSR.APL......S.......P........L.. 123
```

FIG. 3B

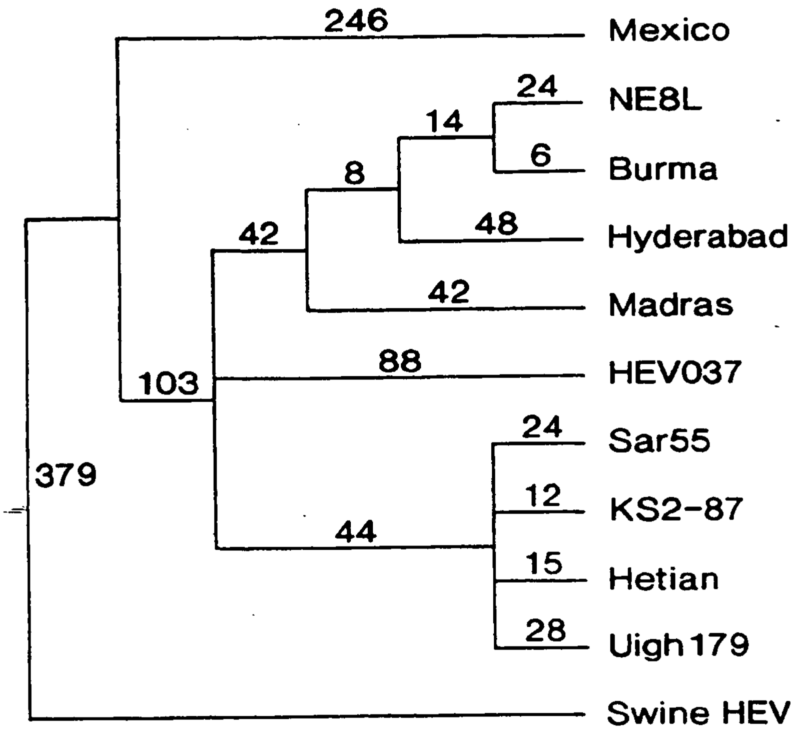

246
Mexico
24
NE8L
14
6
Burma
8
48
Hyderabad
42
42
Madras
103
88
HEV037
24
Sar55
379
12
KS2-87
44
15
Hetian
28
Uigh179
Swine HEV

FIG. 4

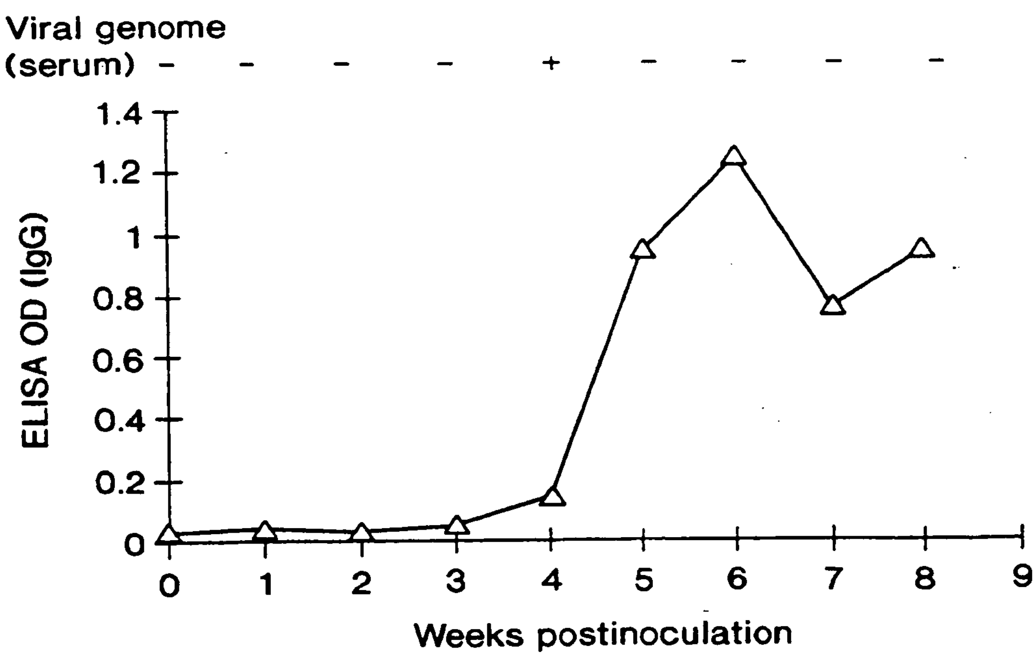

Viral genome
(serum) - - - - + - - - -
ELISA OD (IgG)
1.4
1.2
1
0.8
0.6
0.4
0.2
0
0 1 2 3 4 5 6 7 8 9
Weeks postinoculation

FIG. 5

ATGGAGGCCC	ATCAGTTCAT	TAAGGCTCCT	GGCATTACTA	40
CTGCCATTGA	GCAGGCTGCT	CTGGCTGCGG	CCAACTCCGC	80
CTTGGCGAAT	GCTGTGGTGG	TTCGGCCGTT	TTTATCTCGT	120
GTACAAACTG	AGATCCTTAT	TAATTTGATG	CAACCCCGGC	160
AGTTGGTTTT	CCGCCCTGAG	GTACTTTGGA	ATCATCCTAT	200
CCAGCGGGCA	ATACATAATG	AACTGGAACA	GTACTGCCGA	240
GCCCGGGCTG	GTTGTTGTTT	GGAAGTTGGA	GCCCATCCGA	280
GATTTATTAA	TGACAATCCC	AACGTCCTGC	ACCGGTGCTT	320
CCTTAGACCG	GTTGGCCGAG	ATGTCCAGCG	CTGGTACTCT	360
GCCCCCACCC	GTGGCCCTGC	GGCCAATTGT	CGCCGCTCCG	400
CGCTGCGTGG	CCTTCCCCCC	GTCGACCGCA	CTTACTGTTT	440
TGATGGATTC	TCTCGTTGTG	CTTTCGCTGC	AGAGACCGGT	480
GTGGCCCTCT	ACTCTTTACA	TGACCTTTGG	CCAGCTGATG	520
TTGCGGAGGC	TATGGCCCGC	CACGGGATGA	CACGCCTATA	560
CGCCGCACTG	CACCTTCTTC	CCGAGGTGCT	GCTACCACCC	600
GGCACCTACC	ACACAACTTC	GTACCTCCTG	ATTCACGACG	640
GTGACCGCGC	TGTTGTGACT	TATGAGGGCG	ATACTAGTGC	680
GGGCTATAAC	CATGATGTCT	CCATACTCCG	TGCGTGGATC	720
CGTACCACTA	AAATAGTTGG	TGACCACCCG	TTGGTTATAG	760
AGCGTGTGCG	GGCCATTGGC	TGTCATTTTG	TGCTGCTGCT	800
CACCGCAGCC	CCTGAACCGT	CACCTATGCC	TTATGTCCCC	840
TACCCTCGTT	CAACGGAGGT	GTATGTTCGA	TCCATATTTG	880
GCCCTGGCGG	CTCCCCATCC	TTGTTTCCGT	CAGCCTGCTC	920
TACTAAATCT	ACATTTCATG	CTGTCCCGGT	TCATATCTGG	960
GATCGGCTCA	TGCTTTTTGG	TGCCACCCTG	GATGACCAGG	1000
CCTTTTGTTG	TTCACGGCTC	ATGACCTACC	TCCGTGGTAT	1040
TAGCTACAAG	GTCACTGTCG	GTGCGCTTGT	CGCTAATGAG	1080
GGGTGGAACG	CCTCTGAAGA	TGCTCTTACT	GCAGTGATTA	1120
CTGCCGCTTA	TCTGACTATT	TGCCATCAGC	GTTATCTTCG	1160
CACCCAGGCG	ATATCCAAGG	GCATGCGCCG	GCTGGAGGTT	1200
GAGCACGCCC	AGAAATTTAT	CACAAGACTT	TACAGTTGGC	1240
TATTTGAGAA	GTCTGGCCGT	GATTATATCC	CCGGCCGTCA	1280
GCTTCAGTTC	TACGCACAGT	GCCGGCGGTG	GTTATCTGCA	1320
GGCTTCCACC	TAGACCCCAG	GGTGCTTGTT	TTTGATGAAT	1360
CAGTGCCATG	CCGCTGCAGG	ACGTTTTTGA	AGAAAGTCGC	1400
AGGTAAGTTC	TGCTGTTTTA	TGCGGTGGTT	AGGGCAGGAG	1440
TGTACCTGTT	TCTTGGAGCC	AGCCGAAGGC	TTGGTTGGCG	1480
ACTATGGCCA	TGACAACGAG	GCCTATGAGG	GTTCTGAGGT	1520
CGACCCGGCT	GAACCTGCTC	ATCTTGATGT	TTCTGGGACC	1560
TATGCCGTTC	ACGGGCGCCA	GCTTGAGGCT	CTCTATAGGG	1600
CACTTAATGT	CCCACATGAC	ATCGCCGCTC	GAGCCTCCCG	1640
CCTAACGGCT	ACTGTTGAGC	TCACTGCAAG	CCCAGACCGT	1680
TTAGAGTGCC	GCACTGTGCT	TGGTAATAAG	ACCTTCAGGA	1720
CGACGGTGGT	TGATGGCGCC	CATCTTGAGG	CGAATGGTCC	1760
TGAGCAGTAT	GTCCTATCAT	TCGACGCCTC	CCGCCAGTCT	1800
ATGGGGGCCG	GGTCACATAG	CCTCACTTAT	GAGCTCACCC	1840
CTGCCGGCCT	GCAGGTCAGG	ATTTCATCTA	ATGGCCTGGA	1880

FIG. 6A

TTGCACAGCC	ACATTCCCCC	CCGGCGGCGC	CCCTAGCGCT	1920
GCGCCGGGGG	AGGTGGCGGC	CTTTTGCAGT	GCCCTTTATA	1960
GATATAATAG	GTTCACCCAG	CGGCATTCGC	TGACCGGTGG	2000
GTTATGGCTA	CACCCTGAGG	GATTGCTGGG	CATCTTCCCC	2040
CCTTTCTCCC	CTGGGCACAT	TTGGGAGCCT	GCTAACCCTT	2080
TCTGCGGGGA	GGGGACTTTG	TATACCCGGA	CTTGGTCAAC	2120
ATCTGGCTTT	TCTAGCGATT	TCTCCCCCCC	TGAGGCGGCC	2160
GCCCCCGTTT	TGGCCGCTGC	CCCGGGGCTG	CCCCACCCTA	2200
CCCCACCTGT	TAGTGACATT	TGGGTGTTAC	CACCACCTTC	2240
AAAGGAGTCT	CAGGTCGATG	CGGCATCTGT	GCCCCCTGCT	2280
CCTGAGCCCG	CTGGATTACC	CAGCTCCATT	GTGCTTACCC	2320
TCCCCCCCCC	CCTCCCTCCT	GTGCGTAAGC	CACCAACACC	2360
CCCGCCTTCC	CGCACTCGTC	GTCTCCTCTA	CACCTATCCC	2400
GACGGCGCAA	AGGTGTATGC	GGGGTCATTG	TTTGAATCAG	2440
ACTGTAACTG	GCTGGTTAAT	GCCTCAAACC	CGGGCCACCG	2480
CCCTGGAGGT	GGCCTCTGCC	ATGCTTTTTA	CCAACGTTTC	2520
CCAGAGGCGT	TTTACCCGAC	TGAGTTCATT	ATGCGTGAGG	2560
GCCTTGCAGC	ATATACCCTG	ACCCCGCGCC	CTATCATTCA	2600
TGCAGTGGCC	CCCGACTATA	GGGTTGAGCA	GAATCCGAAG	2640
AGGCTTGAGG	CAGCGTACCG	GGAGACTTGC	TCCCGTCGTG	2680
GCACCGCCGC	CTACCCGCTT	CTAGGCTCGG	GTATATACCA	2720
GGTCCCTGTC	AGCCTCAGTT	TTGATGCCTG	GGAACGCAAT	2760
CACCGCCCCG	GCGATGAGCT	TTATTTGACT	GAGCCCGCCG	2800
CAGCCTGGTT	CGAGGCTAAT	AAGCCGGCGC	AGCCGGCGCT	2840
TACTATAACT	GAGGACACAG	CCCGTACGGC	CAACCTAGCG	2880
TTAGAGATCG	ATGCTGCCAC	AGATGTTGGC	CGTGCTTGTG	2920
CCGGCTGCAC	TATCAGTCCT	GGGATTGTGC	ACTATCAGTT	2960
CACTGCCGGG	GTCCCAGGCT	CGGGCAAGTC	TCGGTCCATA	3000
CAACAGGGAG	ATGTCGATGT	GGTGGTCGTG	CCCACCCGGG	3040
AGCTCCGTAA	TAGTTGGCGT	CGCCGGGGTT	TTGCGGCTTT	3080
CACACCTCAC	ACAGCAGCCC	GTGTCACTAT	CGGTCGCCGC	3120
GTTGTGATTG	ATGAGGCTCC	ATCTCTCCCT	CCACACCTGT	3160
TGCTGTTACA	CATGCAGCGG	GCCTCCTCGG	TCCATCTCCT	3200
TGGTGACCCA	AATCAGATCC	CTGCCATTGA	TTTTGAACAC	3240
GCCGGCCTGG	TTCCCGCGAT	CCGCCCTGAG	CTTGCTCCAA	3280
CGAGCTGGTG	GCACGTTACA	CACCGTTGCC	CGGCCGATGT	3320
ATGCGAGCTC	ATACGCGGAG	CCTACCCTAA	AATCCAGACC	3360
ACGAGCCGTG	TGCTGCGGTC	CCTGTTCTGG	AACGAACCTG	3400
CTATCGGCCA	GAAGTTGGTC	TTCACGCAGG	CTGCTAAAGC	3440
TGCTAACCCT	GGTGCGATTA	CGGTTCATGA	AGCTCAGGGT	3480
GCCACTTTTA	CAGAGACCAC	AATTATAGCC	ACGGCCGATG	3520
CCAGGGGCCT	TATCCAGTCA	TCCCGGGCTC	ACGCTATAGT	3560
CGCACTCACC	CGCCACACTG	AGAAGTGTGT	TATTCTGGAT	3600
GCTCCCGGCC	TGCTGCGTGA	GGTCGGCATT	TCGGATGTGA	3640
TTGTCAATAA	CTTTTTCCTT	GCTGGCGGAG	AAGTCGGCCA	3680
TCACCGCCCT	TCTGTGATAC	CCCGCGGTAA	CCCTGATCAG	3720
AACCTCGGGA	CTCTACAGGC	CTTCCCGCCG	TCCTGCCAGA	3760

FIG. 6B

```
TTAGTGCTTA CCACCAATTG GCTGAAGAAT TAGGCCACCG 3800
TCCGGCTCCT GTTGCCGCCG TCTTGCCCCC TTGCCCTGAG 3840
CTTGAGCAGG GCCTGCTCTA TATGCCACAA GAGCTTACTG 3880
TGTCTGATAG TGTGTTGGTT TTTGAGCTCA CGGATATAGT 3920
CCACTGTCGC ATGGCCGCTC CGAGCCAGCG AAAGGCTGTT 3960
CTCTCAACAC TTGTAGGGAG ATACGGCCGT AGGACGAAAT 4000
TATATGAGGC AGCGCATTCA GATGTTCGTG AGTCCCTGGC 4040
CAGGTTCATT CCCACTATCG GGCCTGTTCA GGCCACCACA 4080
TGTGAGTTGT ATGAGTTGGT TGAGGCCATG GTGGAGAAGG 4120
GACAGGACGG CTCTGCCGTC CTAGAGCTTG ACCTTTGCAA 4160
TCGTGACGTA TCGCGCATCA CATTTTTCCA AAAGGATTGC 4200
AACAAGTTTA CAACTGGTGA GACTATCGCC CATGGCAAGG 4240
TTGGTCAGGG TATATCGGCC TGGAGTAAGA CCTTCTGTGC 4280
TCTGTTTGGC CCGTGGTTCC GTGCCATTGA AAAAGAAATA 4320
CTGGCCCTAC TCCCGCCTAA TATCTTTTAT GGCGACGCCT 4360
ATGAGGAATC AGTGTTCGCT GCCGCTGTGT CCGGGGCGGG 4400
GTCGTGCATG GTATTTGAAA ATGACTTTTC AGAGTTTGAC 4440
AGTACCCAAA ATAATTTCTC CCTTGGCCTT GAGTGTGTGG 4480
TTATGGAGGA GTGCGGCATG CCCCAGTGGC TAATTAGGTT 4520
GTATCATCTG GTTCGGTCAG CCTGGATTTT GCAGGCGCCG 4560
AAGGAGTCTC TTAAGGGTTT CTGGAAGAAG CATTCTGGTG 4600
AGCCTGGTAC CCTTCTCTGG AACACCGTCT GGAACATGGC 4640
GATTATAGCA CATTGTTATG AGTTTCGTGA CTTTCGTGTT 4680
GCCGCCTTCA AGGGTGATGA TTCAGTGGTC CTTTGTAGTG 4720
ACTACCGACA GAGCCGTAAT GCGGCTGCCT TAATTGCAGG 4760
CTGTGGGCTC AAATTGAAGG TTGACTACCG CCCTATTGGG 4800
CTGTATGCCG GGGTGGTGGT GGCCCCTGGT CTGGGGACAC 4840
TGCCTGATGT TGTGCGTTTC GCCGGTCGGT TGTCTGAAAA 4880
GAATTGGGGC CCCGGCCCAG AGCGTGCTGA GCAGCTGCGT 4920
CTTGCTGTTT GTGACTTCCT TCGAGGGTTG ACAAATGTTG 4960
CGCAGGTTTG TGTTGATGTT GTGTCCCGTG TTTATGGAGT 5000
TAGCCCCGGG CTGGTGCATA ACCTTATTGG CATGCTGCAG 5040
ACTATTGCCG ATGGCAAGGC CCACTTTACA GAGACTATTA 5080
AACCTGTGCT TGACCTTACA AACTCTATCA TACAGCGGGT 5120
GGAATGA 5127
```

FIG. 6C

```
Met Glu Ala His Gln Phe Ile Lys Ala Pro Gly Ile
                 5                  10
Thr Thr Ala Ile Glu Gln Ala Ala Leu Ala Ala Ala
         15                  20
Asn Ser Ala Leu Ala Asn Ala Val Val Val Arg Pro
 25                  30                  35
Phe Leu Ser Arg Val Gln Thr Glu Ile Leu Ile Asn
             40                  45
Leu Met Gln Pro Arg Gln Leu Val Phe Arg Pro Glu
     50                  55                  60
Val Leu Trp Asn His Pro Ile Gln Arg Ala Ile His
                 65                  70
Asn Glu Leu Glu Gln Tyr Cys Arg Ala Arg Ala Gly
         75                  80
Cys Cys Leu Glu Val Gly Ala His Pro Arg Phe Ile
 85                  90                  95
Asn Asp Asn Pro Asn Val Leu His Arg Cys Phe Leu
            100                 105
Arg Pro Val Gly Arg Asp Val Gln Arg Trp Tyr Ser
    110                 115                 120
Ala Pro Thr Arg Gly Pro Ala Ala Asn Cys Arg Arg
                125                 130
Ser Ala Leu Arg Gly Leu Pro Pro Val Asp Arg Thr
        135                 140
Tyr Cys Phe Asp Gly Phe Ser Arg Cys Ala Phe Ala
145                 150                 155
Ala Glu Thr Gly Val Ala Leu Tyr Ser Leu His Asp
            160                 165
Leu Trp Pro Ala Asp Val Ala Glu Ala Met Ala Arg
    170                 175                 180
His Gly Met Thr Arg Leu Tyr Ala Ala Leu His Leu
                185                 190
Leu Pro Glu Val Leu Leu Pro Pro Gly Thr Tyr His
        195                 200
Thr Thr Ser Tyr Leu Leu Ile His Asp Gly Asp Arg
205                 210                 215
Ala Val Val Thr Tyr Glu Gly Asp Thr Ser Ala Gly
        220                 225
Tyr Asn His Asp Val Ser Ile Leu Arg Ala Trp Ile
230                 235                 240
Arg Thr Thr Lys Ile Val Gly Asp His Pro Leu Val
            245                 250
Ile Glu Arg Val Arg Ala Ile Gly Cys His Phe Val
    255                 260                 265
Leu Leu Leu Thr Ala Ala Pro Glu Pro Ser Pro Met
                270                 275
```

FIG. 6D

Pro Tyr Val Pro Tyr Pro Arg Ser Thr Glu Val Tyr
280 285
Val Arg Ser Ile Phe Gly Pro Gly Gly Ser Pro Ser
290 295 300
Leu Phe Pro Ser Ala Cys Ser Thr Lys Ser Thr Phe
305 310
His Ala Val Pro Val His Ile Trp Asp Arg Leu Met
315 320 325
Leu Phe Gly Ala Thr Leu Asp Asp Gln Ala Phe Cys
330 335
Cys Ser Arg Leu Met Thr Tyr Leu Arg Gly Ile Ser
340 345
Tyr Lys Val Thr Val Gly Ala Leu Val Ala Asn Glu
350 355 360
Gly Trp Asn Ala Ser Glu Asp Ala Leu Thr Ala Val
365 370
Ile Thr Ala Ala Tyr Leu Thr Ile Cys His Gln Arg
375 380 385
Tyr Leu Arg Thr Gln Ala Ile Ser Lys Gly Met Arg
390 395
Arg Leu Glu Val Glu His Ala Gln Lys Phe Ile Thr
400 405
Arg Leu Tyr Ser Trp Leu Pne Glu Lys Ser Gly Arg
410 415 420
Asp Tyr Ile Pro Gly Arg Gln Leu Gln Phe Tyr Ala
425 430
Gln Cys Arg Arg Trp Leu Ser Ala Gly Phe His Leu
435 440 445
Asp Pro Arg Val Leu Val Phe Asp Glu Ser Val Pro
450 455
Cys Arg Cys Arg Thr Phe Leu Lys Lys Val Ala Gly
460 465
Lys Phe Cys Cys Phe Met Arg Trp Leu Gly Gln Glu
470 475 480
Cys Thr Cys Phe Leu Glu Pro Ala Glu Gly Leu Val
485 490
Gly Asp Tyr Gly His Asp Asn Glu Ala Tyr Glu Gly
495 500 505
Ser Glu Val Asp Pro Ala Glu Pro Ala His Leu Asp
510 515
Val Ser Gly Thr Tyr Ala Val His Gly Arg Gln Leu
520 525
Glu Ala Leu Tyr Arg Ala Leu Asn Val Pro His Asp
530 535 540
Ile Ala Ala Arg Ala Ser Arg Leu Thr Ala Thr Val
545 550
Glu Leu Thr Ala Ser Pro Asp Arg Leu Glu Cys Arg

FIG. 6E

555 560 565
Thr Val Leu Gly Asn Lys Thr Phe Arg Thr Thr Val
570 575
Val Asp Gly Ala His Leu Glu Ala Asn Gly Pro Glu
580 585
Gln Tyr Val Leu Ser Phe Asp Ala Ser Arg Gln Ser
590 595 600
Met Gly Ala Gly Ser His Ser Leu Thr Tyr Glu Leu
605 610
Thr Pro Ala Gly Leu Gln Val Arg Ile Ser Ser Asn
615 620 625
Gly Leu Asp Cys Thr Ala Thr Phe Pro Pro Gly Gly
630 635
Ala Pro Ser Ala Ala Pro Gly Glu Val Ala Ala Phe
640 645
Cys Ser Ala Leu Tyr Arg Tyr Asn Arg Phe Thr Gln
650 655 660
Arg His Ser Leu Thr Gly Gly Leu Trp Leu His Pro
665 670
Glu Gly Leu Leu Gly Ile Phe Pro Pro Phe Ser Pro
675 680 685
Gly His Ile Trp Glu Pro Ala Asn Pro Phe Cys Gly
690 695
Glu Gly Thr Leu Tyr Thr Arg Thr Trp Ser Thr Ser
700 705
Gly Phe Ser Ser Asp Phe Ser Pro Pro Glu Ala Ala
710 715 720
Ala Pro Val Leu Ala Ala Ala Pro Gly Leu Pro His
725 730
Pro Thr Pro Pro Val Ser Asp Ile Trp Val Leu Pro
735 740 745
Pro Pro Ser Lys Glu Ser Gln Val Asp Ala Ala Ser
750 755
Val Pro Pro Ala Pro Glu Pro Ala Gly Leu Pro Ser
760 765
Ser Ile Val Leu Thr Leu Pro Pro Pro Leu Pro Pro
770 775 780
Val Arg Lys Pro Pro Thr Pro Pro Pro Ser Arg Thr
785 790
Arg Arg Leu Leu Tyr Thr Tyr Pro Asp Gly Ala Lys
795 800 805
Val Tyr Ala Gly Ser Leu Phe Glu Ser Asp Cys Asn
810 815
Trp Leu Val Asn Ala Ser Asn Pro Gly His Arg Pro
820 825
Gly Gly Gly Leu Cys His Ala Phe Tyr Gln Arg Phe
830 835 840

FIG. 6F

Pro Glu Ala Phe Tyr Pro Thr Glu Phe Ile Met Arg
845 850
Glu Gly Leu Ala Ala Tyr Thr Leu Thr Pro Arg Pro
855 860 865
Ile Ile His Ala Val Ala Pro Asp Tyr Arg Val Glu
870 875
Gln Asn Pro Lys Arg Leu Glu Ala Ala Tyr Arg Glu
880 885
Thr Cys Ser Arg Arg Gly Thr Ala Ala Tyr Pro Leu
890 895 900
Leu Gly Ser Gly Ile Tyr Gln Val Pro Val Ser Leu
905 910
Ser Phe Asp Ala Trp Glu Arg Asn His Arg Pro Gly
915 920 925
Asp Glu Leu Tyr Leu Thr Glu Pro Ala Ala Ala Trp
930 935
Phe Glu Ala Asn Lys Pro Ala Gln Pro Ala Leu Thr
940 945
Ile Thr Glu Asp Thr Ala Arg Thr Ala Asn Leu Ala
950 955 960
Leu Glu Ile Asp Ala Ala Thr Asp Val Gly Arg Ala
965 970
Cys Ala Gly Cys Thr Ile Ser Pro Gly Ile Val His
975 980 985
Tyr Gln Phe Thr Ala Gly Val Pro Gly Ser Gly Lys
990 995
Ser Arg Ser Ile Gln Gln Gly Asp Val Asp Val Val
1000 1005
Val Val Pro Thr Arg Glu Leu Arg Asn Ser Trp Arg
1010 1015 1020
Arg Arg Gly Phe Ala Ala Phe Thr Pro His Thr Ala
1025 1030
Ala Arg Val Thr Ile Gly Arg Arg Val Val Ile Asp
1035 1040 1045
Glu Ala Pro Ser Leu Pro Pro His Leu Leu Leu Leu
1050 1055
His Met Gln Arg Ala Ser Ser Val His Leu Leu Gly
1060 1065
Asp Pro Asn Gln Ile Pro Ala Ile Asp Phe Glu His
1070 1075 1080
Ala Gly Leu Val Pro Ala Ile Arg Pro Glu Leu Ala
1085 1090
Pro Thr Ser Trp Trp His Val Thr His Arg Cys Pro
1095 1100 1105
Ala Asp Val Cys Glu Leu Ile Arg Gly Ala Tyr Pro
1110 1115
Lys Ile Gln Thr Thr Ser Arg Val Leu Arg Ser Leu

FIG. 6G

1120 1125
Phe Trp Asn Glu Pro Ala Ile Gly Gln Lys Leu Val
1130 1135 1140
Phe Thr Gln Ala Ala Lys Ala Ala Asn Pro Gly Ala
1145 1150
Ile Thr Val His Glu Ala Gln Gly Ala Thr Phe Thr
1155 1160 1165
Glu Thr Thr Ile Ile Ala Thr Ala Asp Ala Arg Gly
1170 1175
Leu Ile Gln Ser Ser Arg Ala His Ala Ile Val Ala
1180 1185
Leu Thr Arg His Thr Glu Lys Cys Val Ile Leu Asp
1190 1195 1200
Ala Pro Gly Leu Leu Arg Glu Val Gly Ile Ser Asp
1205 1210
Val Ile Val Asn Asn Phe Phe Leu Ala Gly Gly Glu
1215 1220 1225
Val Gly His His Arg Pro Ser Val Ile Pro Arg Gly
1230 1235
Asn Pro Asp Gln Asn Leu Gly Thr Leu Gln Ala Phe
1240 1245
Pro Pro Ser Cys Gln Ile Ser Ala Tyr His Gln Leu
1250 1255 1260
Ala Glu Glu Leu Gly His Arg Pro Ala Pro Val Ala
1265 1270
Ala Val Leu Pro Pro Cys Pro Glu Leu Glu Gln Gly
1275 1280 1285
Leu Leu Tyr Met Pro Gln Glu Leu Thr Val Ser Asp
1290 1295
Ser Val Leu Val Phe Glu Leu Thr Asp Ile Val His
1300 1305
Cys Arg Met Ala Ala Pro Ser Gln Arg Lys Ala Val
1310 1315 1320
Leu Ser Thr Leu Val Gly Arg Tyr Gly Arg Arg Thr
1325 1330
Lys Leu Tyr Glu Ala Ala His Ser Asp Val Arg Glu
1335 1340 1345
Ser Leu Ala Arg Phe Ile Pro Thr Ile Gly Pro Val
1350 1355
Gln Ala Thr Thr Cys Glu Leu Tyr Glu Leu Val Glu
1360 1365
Ala Met Val Glu Lys Gly Gln Asp Gly Ser Ala Val
1370 1375 1380
Leu Glu Leu Asp Leu Cys Asn Arg Asp Val Ser Arg
1385 1390
Ile Thr Phe Phe Gln Lys Asp Cys Asn Lys Phe Thr
1395 1400 1405

FIG. 6H

Thr Gly Glu Thr Ile Ala His Gly Lys Val Gly Gln
1410 1415
Gly Ile Ser Ala Trp Ser Lys Thr Phe Cys Ala Leu
1420 1425
Phe Gly Pro Trp Phe Arg Ala Ile Glu Lys Glu Ile
1430 1435 1440
Leu Ala Leu Leu Pro Pro Asn Ile Phe Tyr Gly Asp
1445 1450
Ala Tyr Glu Glu Ser Val Phe Ala Ala Ala Val Ser
1455 1460 1465
Gly Ala Gly Ser Cys Met Val Phe Glu Asn Asp Phe
1470 1475
Ser Glu Phe Asp Ser Thr Gln Asn Asn Phe Ser Leu
1480 1485
Gly Leu Glu Cys Val Val Met Glu Glu Cys Gly Met
1490 1495 1500
Pro Gln Trp Leu Ile Arg Leu Tyr His Leu Val Arg
1505 1510
Ser Ala Trp Ile Leu Gln Ala Pro Lys Glu Ser Leu
1515 1520 1525
Lys Gly Phe Trp Lys Lys His Ser Gly Glu Pro Gly
1530 1535
Thr Leu Leu Trp Asn Thr Val Trp Asn Met Ala Ile
1540 1545
Ile Ala His Cys Tyr Glu Phe Arg Asp Phe Arg Val
1550 1555 1560
Ala Ala Phe Lys Gly Asp Asp Ser Val Val Leu Cys
1565 1570
Ser Asp Tyr Arg Gln Ser Arg Asn Ala Ala Ala Leu
1575 1580 1585
Ile Ala Gly Cys Gly Leu Lys Leu Lys Val Asp Tyr
1590 1595
Arg Pro Ile Gly Leu Tyr Ala Gly Val Val Val Ala
1600 1605
Pro Gly Leu Gly Thr Leu Pro Asp Val Val Arg Phe
1610 1615 1620
Ala Gly Arg Leu Ser Glu Lys Asn Trp Gly Pro Gly
1625 1630
Pro Glu Arg Ala Glu Gln Leu Arg Leu Ala Val Cys
1635 1640 1645
Asp Phe Leu Arg Gly Leu Thr Asn Val Ala Gln Val
1650 1655
Cys Val Asp Val Val Ser Arg Val Tyr Gly Val Ser
1660 1665
Pro Gly Leu Val His Asn Leu Ile Gly Met Leu Gln
1670 1675 1680
Thr Ile Ala Asp Gly Lys Ala His Phe Thr Glu Thr

FIG. 6I

```
                1685                    1690
Ile Lys Pro Val Leu Asp Leu Thr Asn Ser Ile Ile
    1695                1700                1705
Gln Arg Val Glu
            1709
```

FIG. 6J

TTCGATGCCA TGGAGGCCCA TCAGTTCATT AAGGCTCCTG 40
GCATTACTAC TGCCATTGAG CAGGCTGCTC TGGCTGCGGC 80
CAACTCCGCC TTGGCGAATG CTGTGGTGGT TCGGCCGTTT 120
TTATCTCGTG TACAAACTGA GATCCTTATT AATTTGATGC 160
AACCCCGGCA GTTGGTTTTC CGCCCTGAGG TACTTTGGAA 200
TCATCCTATC CAGCGGGCAA TACATAATGA ACTGGAACAG 240
TACTGCCGAG CCCGGGCTGG TTGTTGTTTG GAAGTTGGAG 280
CCCATCCGAG ATTTATTAAT GACAATCCCA ACGTCCTGCA 320
CCGGTGCTTC CTTAGACCGG TTGGCCGAGA TGTCCAGCGC 360
TGGTACTCTG CCCCCACCCG TGGCCCTGCG GCCAATTGTC 400
GCCGCTCCGC GCTGCGTGGC CTTCCCCCCG TCGACCGCAC 440
TTACTGTTTT GATGGATTCT CTCGTTGTGC TTTCGCTGCA 480
GAGACCGGTG TGGCCCTCTA CTCTTTACAT GACCTTTGGC 520
CAGCTGATGT TGCGGAGGCT ATGGCCCGCC ACGGGATGAC 560
ACGCCTATAC GCCGCACTGC ACCTTCTTCC CGAGGTGCTG 600
CTACCACCCG GCACCTACCA CACAACTTCG TACCTCCTGA 640
TTCACGACGG TGACCGCGCT GTTGTGACTT ATGAGGGCGA 680
TACTAGTGCG GGCTATAACC ATGATGTCTC CATACTCCGT 720
GCGTGGATCC GTACCACTAA AATAGTTGGT GACCACCCGT 760
TGGTTATAGA GCGTGTGCGG GCCATTGGCT GTCATTTTGT 800
GCTGCTGCTC ACCGCAGCCC CTGAACCGTC ACCTATGCCT 840
TATGTCCCCT ACCCTCGTTC AACGGAGGTG TATGTTCGAT 880
CCATATTTGG CCCTGGCGGC TCCCCATCCT TGTTTCCGTC 920
AGCCTGCTCT ACTAAATCTA CATTTCATGC TGTCCCGGTT 960
CATATCTGGG ATCGGCTCAT GCTTTTTGGT GCCACCCTGG 1000
ATGACCAGGC CTTTTGTTGT TCACGGCTCA TGACCTACCT 1040
CCGTGGTATT AGCTACAAGG TCACTGTCGG TGCGCTTGTC 1080
GCTAATGAGG GGTGGAACGC CTCTGAAGAT GCTCTTACTG 1120
CAGTGATTAC TGCCGCTTAT CTGACTATTT GCCATCAGCG 1160
TTATCTTCGC ACCCAGGCGA TATCCAAGGG CATGCGCCGG 1200
CTGGAGGTTG AGCACGCCCA GAAATTTATC ACAAGACTTT 1240
ACAGTTGGCT ATTTGAGAAG TCTGGCCGTG ATTATATCCC 1280
CGGCCGTCAG CTTCAGTTCT ACGCACAGTG CCGGCGGTGG 1320
TTATCTGCAG GCTTCCACCT AGACCCCAGG GTGCTTGTTT 1360
TTGATGAATC AGTGCCATGC CGCTGCAGGA CGTTTTTGAA 1400
GAAAGTCGCA GGTAAGTTCT GCTGTTTTAT GCGGTGGTTA 1440
GGGCAGGAGT GTACCTGTTT CTTGGAGCCA GCCGAAGGCT 1480
TGGTTGGCGA CTATGGCCAT GACAACGAGG CCTATGAGGG 1520
TTCTGAGGTC GACCCGGCTG AACCTGCTCA TCTTGATGTT 1560
TCTGGGACCT ATGCCGTTCA CGGGCGCCAG CTTGAGGCTC 1600
TCTATAGGGC ACTTAATGTC CCACATGACA TCGCCGCTCG 1640
AGCCTCCCGC CTAACGGCTA CTGTTGAGCT CACTGCAAGC 1680
CCAGACCGTT TAGAGTGCCG CACTGTGCTT GGTAATAAGA 1720
CCTTCAGGAC GACGGTGGTT GATGGCGCCC ATCTTGAGGC 1760
GAATGGTCCT GAGCAGTATG TCCTATCATT CGACGCCTCC 1800
CGCCAGTCTA TGGGGGCCGG GTCACATAGC CTCACTTATG 1840
AGCTCACCCC TGCCGGCCTG CAGGTCAGGA TTTCATCTAA 1880

FIG. 7A

TGGCCTGGAT	TGCACAGCCA	CATTCCCCCC	CGGCGGCGCC	1920
CCTAGCGCTG	CGCCGGGGGA	GGTGGCGGCC	TTTTGCAGTG	1960
CCCTTTATAG	ATATAATAGG	TTCACCCAGC	GGCATTCGCT	2000
GACCGGTGGG	TTATGGCTAC	ACCCTGAGGG	ATTGCTGGGC	2040
ATCTTCCCCC	CTTTCTCCCC	TGGGCACATT	TGGGAGCCTG	2080
CTAACCCTTT	CTGCGGGGAG	GGGACTTTGT	ATACCCGGAC	2120
TTGGTCAACA	TCTGGCTTTT	CTAGCGATTT	CTCCCCCCCT	2160
GAGGCGGCCG	CCCCCGTTTT	GGCCGCTGCC	CCGGGGCTGC	2200
CCCACCCTAC	CCCACCTGTT	AGTGACATTT	GGGTGTTACC	2240
ACCACCTTCA	AAGGAGTCTC	AGGTCGATGC	GGCATCTGTG	2280
CCCCCTGCTC	CTGAGCCCGC	TGGATTACCC	AGCTCCATTG	2320
TGCTTACCCT	CCCCCCCCCC	CTCCCTCCTG	TGCGTAAGCC	2360
ACCAACACCC	CCGCCTTCCC	GCACTCGTCG	TCTCCTCTAC	2400
ACCTATCCCG	ACGGCGCAAA	GGTGTATGCG	GGGTCATTGT	2440
TTGAATCAGA	CTGTAACTGG	CTGGTTAATG	CCTCAAACCC	2480
GGGCCACCGC	CCTGGAGGTG	GCCTCTGCCA	TGCTTTTTAC	2520
CAACGTTTCC	CAGAGGCGTT	TTACCCGACT	GAGTTCATTA	2560
TGCGTGAGGG	CCTTGCAGCA	TATACCCTGA	CCCCGCGCCC	2600
TATCATTCAT	GCAGTGGCCC	CCGACTATAG	GGTTGAGCAG	2640
AATCCGAAGA	GGCTTGAGGC	AGCGTACCGG	GAGACTTGCT	2680
CCCGTCGTGG	CACCGCCGCC	TACCCGCTTC	TAGGCTCGGG	2720
TATATACCAG	GTCCCTGTCA	GCCTCAGTTT	TGATGCCTGG	2760
GAACGCAATC	ACCGCCCCGG	CGATGAGCTT	TATTTGACTG	2800
AGCCCGCCGC	AGCCTGGTTC	GAGGCTAATA	AGCCGGCGCA	2840
GCCGGCGCTT	ACTATAACTG	AGGACACAGC	CCGTACGGCC	2880
AACCTAGCGT	TAGAGATCGA	TGCTGCCACA	GATGTTGGCC	2920
GTGCTTGTGC	CGGCTGCACT	ATCAGTCCTG	GGATTGTGCA	2960
CTATCAGTTC	ACTGCCGGGG	TCCCAGGCTC	GGGCAAGTCT	3000
CGGTCCATAC	AACAGGGAGA	TGTCGATGTG	GTGGTCGTGC	3040
CCACCCGGGA	GCTCCGTAAT	AGTTGGCGTC	GCCGGGGTTT	3080
TGCGGCTTTC	ACACCTCACA	CAGCAGCCCG	TGTCACTATC	3120
GGTCGCCGCG	TTGTGATTGA	TGAGGCTCCA	TCTCTCCCTC	3160
CACACCTGTT	GCTGTTACAC	ATGCAGCGGG	CCTCCTCGGT	3200
CCATCTCCTT	GGTGACCCAA	ATCAGATCCC	TGCCATTGAT	3240
TTTGAACACG	CCGGCCTGGT	TCCCGCGATC	CGCCCTGAGC	3280
TTGCTCCAAC	GAGCTGGTGG	CACGTTACAC	ACCGTTGCCC	3320
GGCCGATGTA	TGCGAGCTCA	TACGCGGAGC	CTACCCTAAA	3360
ATCCAGACCA	CGAGCCGTGT	GCTGCGGTCC	CTGTTCTGGA	3400
ACGAACCTGC	TATCGGCCAG	AAGTTGGTCT	TCACGCAGGC	3440
TGCTAAAGCT	GCTAACCCTG	GTGCGATTAC	GGTTCATGAA	3480
GCTCAGGGTG	CCACTTTTAC	AGAGACCACA	ATTATAGCCA	3520
CGGCCGATGC	CAGGGGCCTT	ATCCAGTCAT	CCCGGGCTCA	3560
CGCTATAGTC	GCACTCACCC	GCCACACTGA	GAAGTGTGTT	3600
ATTCTGGATG	CTCCCGGCCT	GCTGCGTGAG	GTCGGCATTT	3640
CGGATGTGAT	TGTCAATAAC	TTTTTCCTTG	CTGGCGGAGA	3680
AGTCGGCCAT	CACCGCCCTT	CTGTGATACC	CCGCGGTAAC	3720
CCTGATCAGA	ACCTCGGGAC	TCTACAGGCC	TTCCCGCCGT	3760

FIG. 7B

CCTGCCAGAT TAGTGCTTAC CACCAATTGG CTGAAGAATT 3800
AGGCCACCGT CCGGCTCCTG TTGCCGCCGT CTTGCCCCCT 3840
TGCCCTGAGC TTGAGCAGGG CCTGCTCTAT ATGCCACAAG 3880
AGCTTACTGT GTCTGATAGT GTGTTGGTTT TTGAGCTCAC 3920
GGATATAGTC CACTGTCGCA TGGCCGCTCC GAGCCAGCGA 3960
AAGGCTGTTC TCTCAACACT TGTAGGGAGA TACGGCCGTA 4000
GGACGAAATT ATATGAGGCA GCGCATTCAG ATGTTCGTGA 4040
GTCCCTGGCC AGGTTCATTC CCACTATCGG GCCTGTTCAG 4080
GCCACCACAT GTGAGTTGTA TGAGTTGGTT GAGGCCATGG 4120
TGGAGAAGGG ACAGGACGGC TCTGCCGTCC TAGAGCTTGA 4160
CCTTTGCAAT CGTGACGTAT CGCGCATCAC ATTTTTCCAA 4200
AAGGATTGCA ACAAGTTTAC AACTGGTGAG ACTATCGCCC 4240
ATGGCAAGGT TGGTCAGGGT ATATCGGCCT GGAGTAAGAC 4280
CTTCTGTGCT CTGTTTGGCC CGTGGTTCCG TGCCATTGAA 4320
AAAGAAATAC TGGCCCTACT CCCGCCTAAT ATCTTTTATG 4360
GCGACGCCTA TGAGGAATCA GTGTTCGCTG CCGCTGTGTC 4400
CGGGGCGGGG TCGTGCATGG TATTTGAAAA TGACTTTTCA 4440
GAGTTTGACA GTACCCAAAA TAATTTCTCC CTTGGCCTTG 4480
AGTGTGTGGT TATGGAGGAG TGCGGCATGC CCCAGTGGCT 4520
AATTAGGTTG TATCATCTGG TTCGGTCAGC CTGGATTTTG 4560
CAGGCGCCGA AGGAGTCTCT TAAGGGTTTC TGGAAGAAGC 4600
ATTCTGGTGA GCCTGGTACC CTTCTCTGGA ACACCGTCTG 4640
GAACATGGCG ATTATAGCAC ATTGTTATGA GTTTCGTGAC 4680
TTTCGTGTTG CCGCCTTCAA GGGTGATGAT TCAGTGGTCC 4720
TTTGTAGTGA CTACCGACAG AGCCGTAATG CGGCTGCCTT 4760
AATTGCAGGC TGTGGGCTCA AATTGAAGGT TGACTACCGC 4800
CCTATTGGGC TGTATGCCGG GGTGGTGGTG GCCCCTGGTC 4840
TGGGGACACT GCCTGATGTT GTGCGTTTCG CCGGTCGGTT 4880
GTCTGAAAAG AATTGGGGCC CCGGCCCAGA GCGTGCTGAG 4920
CAGCTGCGTC TTGCTGTTTG TGACTTCCTT CGAGGGTTGA 4960
CAAATGTTGC GCAGGTTTGT GTTGATGTTG TGTCCCGTGT 5000
TTATGGAGTT AGCCCCGGGC TGGTGCATAA CCTTATTGGC 5040
ATGCTGCAGA CTATTGCCGA TGGCAAGGCC CACTTTACAG 5080
AGACTATTAA ACCTGTGCTT GACCTTACAA ACTCTATCAT 5120
ACAGCGGGTG GAATGAATAA CATGTCTTTT GCATCGCCCA 5160
TGGGATCACC ATGCGCCCTA GGGCTGTTCT GTTGTTGCTC 5200
TTCGTGCTTC TGCCTATGCT GCCCGCGCCA CCGGCCGGCC 5240
AGCCGTCTGG CCGCCGTTGT GGGCGGCGCA ACGGCGGTGC 5280
CGGCGGTGGT TTCTGGGGTG ACAGGGTTGA TTCTCAGCCC 5320
TTCGCCCTCC CCTATATTCA TCCAACCAAC CCCTTCGCTG 5360
CCGATGTCGT TTCACAACCC GGGGCTGGAG TTCGCCCTCG 5400
ACAGCCGCCC CGCCCCCTTG GCTCCGCTTG GCGTGACCAG 5440
TCCCAGCGCC CCTCCACTGC CCCCCGTCGT CGATCTGCCC 5480
CAGCTGGGGC TGCGCCGCTG ACTGCTGTAT CACCGGCCCC 5520
CGACACAGCT CCTGTACCTG ATGTTGACTC ACGTGGTGCT 5560
ATCCTGCGCC GGCAGTACAA TCTGTCTACG TCCCCGCTCA 5600
CGTCATCTGT CGCTGCTGGT ACCAACCTGG TTCTCTATGC 5640

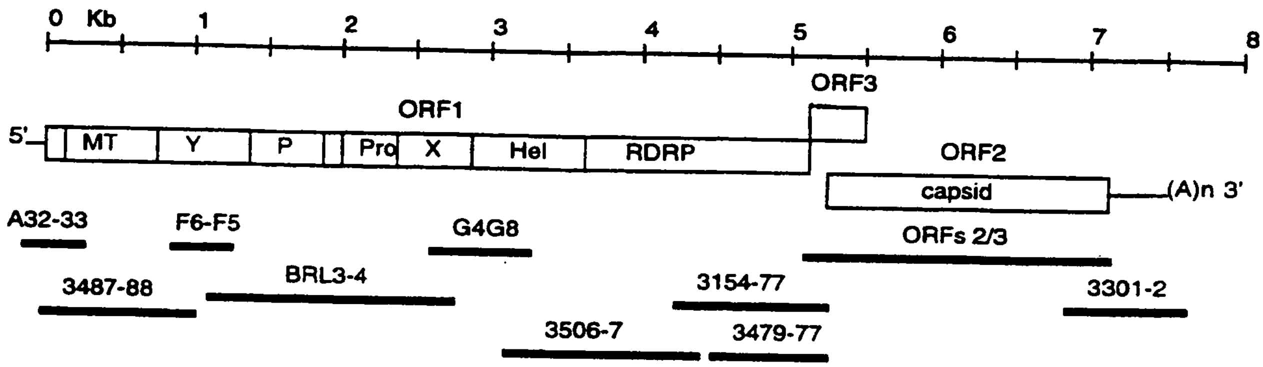
FIG. 7C

FIGURE 7D

```
CGCCCCGCTG AATCCTCTCT TGCCCCTCCA GGATGGCACC 5680
AACACTCATA TTATGGCTAC TGAGGCGTCC AATTATGCTC 5720
AGTATCGGGT TGTTCGAGCT ACGATCCGTT ATCGCCCGCT 5760
GGTGCCAAAT GCTGTTGGTG GCTATGCTAT CTCTATTTCT 5800
TTCTGGCCTC AAACTACAAC CACCCCTACT TCAGTTGACA 5840
TGAACTCTAT TACCTCCACT GATGTCAGGA TTTTGGTTCA 5880
GCCCGGTATT GCCTCCGAGT TAGTCATCCC TAGTGAGCGC 5920
CTTCATTACC GCAATCAAGG CTGGCGCTCT GTAGAGACCA 5960
CGGGCGTGGC CGAGGAGGAA GCTACCTCCG GTCTGGTAAT 6000
GCTTTGCATT CACGGTTCTC CTGTTAACTC CTATACTAAC 6040
ACACCTTACA CTGGTGCATT GGGGCTCCTT GATTTTGCAT 6080
TAGAGCTTGA ATTCAGAAAT TTGACACCCG GGAACACTAA 6120
CACCCGTGTT TCCCGGTACA CCAGCACAGC CCGCCATCGG 6160
CTGCGCCGCG GTGCTGATGG GACCGCAGAG CTTACCACCA 6200
CAGCAGCCAC ACGTTTCATG AAGGACTTGC ATTTCACCGG 6240
CACGAACGGC GTTGGTGAGG TGGGTCGCGG TATAGCTCTA 6280
ACACTGTTTA ACCTTGCTGA TACGCTTCTT GGTGGTTTAC 6320
CGACAGAATT GATTTCGTCG GCCGGGGGCC AACTGTTTTA 6360
CTCCCGCCCT GTCGTCTCGG CCAATGGCGA GCCGACGGTT 6400
AAGTTATATA CATCTGTTGA GAATGCGCAG CAGGACAAGG 6440
GCATTACCAT CCCACACGAT ATAGATCTGG GTGATTCCCG 6480
TGTGGTTATT CAGGATTATG ATAACCAGCA CGAGCAAGAC 6520
CGACCTACTC CGTCACCAGC CCCCTCTCGC CCTTTCTCAG 6560
TTCTTCGCGC CAATGATGTT CTGTGGCTCT CCCTCACCGC 6600
CGCTGAGTAC GATCAGACTA CATATGGGTC GTCCACCAAC 6640
CCTATGTATG TCTCCGATAC GGTCACGCTA GTTAATGTGG 6680
CCACTGGTGC TCAGGCTGTT GCCCGCTCTC TTGATTGGTC 6720
TAAAGTCACT CTGGATGGCC GCCCCCTCAC TACCATTCAG 6760
CAGTATTCAA AGACATTCTA TGTTCTCCCG CTCCGCGGGA 6800
AGCTGTCCTT TTGGGAGGCT GGTACCACTA AGGCCGGCTA 6840
CCCGTATAAT TATAATACCA CTGCTAGTGA TCAAATTTTG 6880
ATTGAGAACG CGGCTGGCCA CCGTGTTGCT ATCTCTACCT 6920
ATACCACTAG CTTGGGTGCC GGCCCTACCT CGATTTCCGC 6960
CGTTGGTGTG CTAGCCCCAC ACTCGGCTCT CGCCGTCCTT 7000
GAGGATACTG TTGATTACCC TGCTCGTGCT CATACTTTTG 7040
ATGATTTCTG CCCGGAGTGC CGCACCCTTG GTTTGCAGGG 7080
TTGTGCATTC CAGTCTACTA TTGCTGAGCT TCAGCGTCTT 7120
AAAATGAAGG TAGGTAAAAC CCGGGAGTCT TAATTAATTC 7160
CTTTTGTGCC CCCTTCATAG CTTCCTTTGG TTTTATTTCT 7200
TATTTCT 7201
```

FIGURE 8

A



B

A32-33
forward, 5'-ATATGTGGTCGATGCCATGGAG-3'; reverse, 5'-CTCGGCAGTACTGTTCCAGTTC-3'
forward, 5'-TCGATGCCATGGAGGCCCATCA-3'; reverse, 5'-GTATTGCCCGCTGGATAGGATG-3'

3487-88
forward, 5'-AAGGCTCC(A)TGGCATCACTACTG-3'; reverse, 5'-CAGAGGCA(G)TTCCAGCCTTCATT-3'
forward, 5'-TGGCATCACTACTGC(T)TATTGAG-3'; reverse, 5'-GGGAGCAGCAAAAGGCT(C)TGGTC-3'

F6-F5
forward, 5'-TCTACATTTCATGCTGTCCCGGTTCATA-3'; reverse, 5'-TCCTGACCAAGCCACTTCAT-3'
forward, 5'-GATGACCAAGCCTTTTGCTG-3'; reverse, 5'-TAATCACGGCCGGACTTCTC-3'

BRL3-4
forward, 5'-TGCCATCAGCGTTATCTTCGCACCCA-3'; reverse, 5'-GCACGGCCAACATCTGTGGCAGCATC-3'
forward, 5'-ACCCAGGCGATATCCAAGGGCATGCG-3'; reverse, 5'-GCATCGATCTCTAACGCTAGGTTGGC-3'

G4G8
forward, 5'-TATA(C)GG(A)TTGGAACATAACCC-3'; reverse, 5'-CGGTGTGTAACGTGCCACCA-3'
forward, 5'-TTT(C)GAC(T)GCCTGGGAGCGGAA-3', reverse, 5'-AAATCAATGGCAGGGATCTG-3'

3506-7
forward, 5'-GGCGC(T)C(A)GGGTTGTCATTGATGA-3'; reverse, 5'-GGGAGTAGGGCCAGTATTTCTT-3'
forward, 5'-TTGGC(T)GACCCGAAT(C)CAGATCCC-3'; reverse, 5'-CTTTTTCAATGGCACGGAACCA-3'

3154-77
forward, 5'-GAGGCC(G)ATGGTC(G)GAGAAGGGCCA-3'; reverse, 5'-AAGAGCAACAACAGAACAGCCC-3'
forward, 5'-ACCTTC(T)TTCCAGAAA(G)GATTGTAA-3'; reverse, 5'-CTAGGGCGCATGGTGATCCCAT-3'

3479-77
forward, 5'-CTGGAAGAAA(G)CAC(T)TCT(C)GGTGAG-3'; reverse, 5'-AAGAGCAACAACAGAACAGCCC-3'
forward, 5'-TGGAATACT(G)GTC(G)TGGAAC(T)ATGG-3'; reverse, 5'-CTAGGGCGCATGGTGATCCCAT-3'

3301-2
forward, 5'-CTCAGTTCTTCGCGCCAATGAT-3'; r verse, 5'-TTTTTTTCAGGGAGCGCGGG(A)AC-3'

A32-33
forward, 5'-ATATGTGGTCGATGCCATGGAG-3'; reverse, 5'-CTCGGCAGTACTGTTCCAGTTC-3'
forward, 5'-TCGATGCCATGGAGGCCCATCA-3'; reverse, 5'-GTATTGCCCGCTGGATAGGATG-3'

3487-88
forward, 5'-AAGGCTCC(A)TGGCATCACTACTG-3'; reverse, 5'-CAGAGGCA(G)TTCCAGCCTTCATT-3'
forward, 5'-TGGCATCACTACTGC(T)TATTGAG-3'; reverse, 5'-GGGAGCAGCAAAAGGCT(C)TGGTC-3'

F6-F5
forward, 5'-TCTACATTTCATGCTGTCCCGGTTCATA-3'; reverse, 5'-TCCTGACCAAGCCACTTCAT-3'
forward, 5'-GATGACCAAGCCTTTTGCTG-3'; reverse, 5'-TAATCACGGCCGGACTTCTC-3'

BRL3-4
forward, 5'-TGCCATCAGCGTTATCTTCGCACCCA-3'; reverse, 5'-GCACGGCCAACATCTGTGGCAGCATC-3'
forward, 5'-ACCCAGGCGATATCCAAGGGCATGCG-3'; reverse, 5'-GCATCGATCTCTAACGCTAGGTTGGC-3'

G4G8
forward, 5'-TATA(C)GG(A)TTGGAACATAACCC-3'; reverse, 5'-CGGTGTGTAACGTGCCACCA-3'
forward, 5'-TTT(C)GAC(T)GCCTGGGAGCGGAA-3', reverse, 5'-AAATCAATGGCAGGGATCTG-3'

3506-7
forward, 5'-GGCGC(T)C(A)GGGTTGTCATTGATGA-3'; reverse, 5'-GGGAGTAGGGCCAGTATTTCTT-3'
forward, 5'-TTGGC(T)GACCCGAAT(C)CAGATCCC-3'; reverse, 5'-CTTTTTCAATGGCACGGAACCA-3'

3154-77
forward, 5'-GAGGCC(G)ATGGTC(G)GAGAAGGGCCA-3'; reverse, 5'-AAGAGCAACAACAGAACAGCCC-3'
forward, 5'-ACCTTC(T)TTCCAGAAA(G)GATTGTAA-3'; reverse, 5'-CTAGGGCGCATGGTGATCCCAT-3'

3479-77
forward, 5'-CTGGAAGAAA(G)CAC(T)TCT(C)GGTGAG-3'; reverse, 5'-AAGAGCAACAACAGAACAGCCC-3'
forward, 5'-TGGAATACT(G)GTC(G)TGGAAC(T)ATGG-3'; reverse, 5'-CTAGGGCGCATGGTGATCCCAT-3'

3301-2
forward, 5'-CTCAGTTCTTCGCGCCAATGAT-3'; reverse, 5'-TTTTTTTCAGGGAGCGCGGG(A)AC-3'

FIG. 8B

PCT

WORLD INTELLECTUAL PROPERTY ORGANIZATION
International Bureau

INTERNATIONAL APPLICATION PUBLISHED UNDER THE PATENT COOPERATION TREATY (PCT)

(51) International Patent Classification 6 :
C12N 15/51, C07K 14/08, A61K 39/29, C12Q 1/68, C07K 16/10

A3

(11) International Publication Number: WO 99/04029

(43) Internati nal Publicati n Date: 28 January 1999 (28.01.99)

(21) International Application Number: PCT/US98/14665

(22) International Filing Date: 17 July 1998 (17.07.98)

(30) Priority Data:
60/053,069 18 July 1997 (18.07.97) US

(63) Related by Continuation (CON) or Continuation-in-Part (CIP) to Earlier Application
US 60/053,069 (CIP)
Filed on 18 July 1997 (18.07.97)

(71) Applicant *(for all designated States except US)*: THE GOVERNMENT OF THE UNITED STATES OF AMERICA, represented by THE SECRETARY, DEPARTMENT OF HEALTH AND HUMAN SERVICES [US/US]; Office of Technology Transfer, National Institutes of Health, Suite 325, 6011 Executive Boulevard, Rockville, MD 20852 (US).

(72) Inventors; and
(75) Inventors/Applicants *(for US only)*: MENG, Xiang-Jin [CN/US]; Apartment 611, 3 Pooks Hill Road, Bethesda, MD 20814 (US). EMERSON, Suzanne, U. [US/US]; 18201 Woodcrest Drive, Rockville, MD 20852 (US). PURCELL, Robert, H. [US/US]; 17517 White Grounds Road, Boyds, MD 28841 (US).

(74) Agents: FEILER, William, S. et al.; Morgan & Finnegan, L.L.P., 345 Park Avenue, New York, NY 10154 (US).

(81) Designated States: AL, AM, AT, AU, AZ, BA, BB, BG, BR, BY, CA, CH, CN, CU, CZ, DE, DK, EE, ES, FI, GB, GE, GH, GM, HR, HU, ID, IL, IS, JP, KE, KG, KP, KR, KZ, LC, LK, LR, LS, LT, LU, LV, MD, MG, MK, MN, MW, MX, NO, NZ, PL, PT, RO, RU, SD, SE, SG, SI, SK, SL, TJ, TM, TR, TT, UA, UG, US, UZ, VN, YU, ZW, ARIPO patent (GH, GM, KE, LS, MW, SD, SZ, UG, ZW), Eurasian patent (AM, AZ, BY, KG, KZ, MD, RU, TJ, TM), European patent (AT, BE, CH, CY, DE, DK, ES, FI, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE), OAPI patent (BF, BJ, CF, CG, CI, CM, GA, GN, GW, ML, MR, NE, SN, TD, TG).

Published
With international search report.

(88) Date of publication of the international search report: 1 April 1999 (01.04.99)

(54) Title: A SWINE HEPATITIS E VIRUS AND USES THEREOF

(57) Abstract

The present invention discloses the isolation and characterization of a novel swine hepatitis E virus and the use of the virus, the proteins and its nucleic acid sequence as diagnostic reagents and vaccines.

FOR THE PURPOSES OF INFORMATION ONLY

Codes used to identify States party to the PCT on the front pages of pamphlets publishing international applications under the PCT.

AL	Albania	ES	Spain	LS	Lesotho	SI	Slovenia
AM	Armenia	FI	Finland	LT	Lithuania	SK	Slovakia
AT	Austria	FR	France	LU	Luxembourg	SN	Senegal
AU	Australia	GA	Gabon	LV	Latvia	SZ	Swaziland
AZ	Azerbaijan	GB	United Kingdom	MC	Monaco	TD	Chad
BA	Bosnia and Herzegovina	GE	Georgia	MD	Republic of Moldova	TG	Togo
BB	Barbados	GH	Ghana	MG	Madagascar	TJ	Tajikistan
BE	Belgium	GN	Guinea	MK	The former Yugoslav Republic of Macedonia	TM	Turkmenistan
BF	Burkina Faso	GR	Greece			TR	Turkey
BG	Bulgaria	HU	Hungary	ML	Mali	TT	Trinidad and Tobago
BJ	Benin	IE	Ireland	MN	Mongolia	UA	Ukraine
BR	Brazil	IL	Israel	MR	Mauritania	UG	Uganda
BY	Belarus	IS	Iceland	MW	Malawi	US	United States of America
CA	Canada	IT	Italy	MX	Mexico	UZ	Uzbekistan
CF	Central African Republic	JP	Japan	NE	Niger	VN	Viet Nam
CG	Congo	KE	Kenya	NL	Netherlands	YU	Yugoslavia
CH	Switzerland	KG	Kyrgyzstan	NO	Norway	ZW	Zimbabwe
CI	Côte d'Ivoire	KP	Democratic People's Republic of Korea	NZ	New Zealand		
CM	Cameroon			PL	Poland		
CN	China	KR	Republic of Korea	PT	Portugal		
CU	Cuba	KZ	Kazakstan	RO	Romania		
CZ	Czech Republic	LC	Saint Lucia	RU	Russian Federation		
DE	Germany	LI	Liechtenstein	SD	Sudan		
DK	Denmark	LK	Sri Lanka	SE	Sweden		
EE	Estonia	LR	Liberia	SG	Singapore		

INTERNATIONAL SEARCH REPORT

International Application No
PCT/US 98/14665

A. CLASSIFICATION OF SUBJECT MATTER
IPC 6 C12N15/51 C07K14/08 A61K39/29 C12Q1/68 C07K16/10

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)
IPC 6 C07K C12N A61K C12Q

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practical, search terms used)

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category °	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
Y	WO 96 10580 A (US HEALTH ;TSAREV SERGEI A (US); EMERSON SUZANNE U (US); PURCELL R) 11 April 1996 see the claims see abstract; examples 1,2,6,8 see page 9 - page 21	1-44
Y	CLAYSON E T ET AL.: "Detection of hepatitis E virus infections among domestic swine in the Katmandu valley of Nepal" AMERICAN JOURNAL OF TROPICAL MEDICINE AND HYGIENE, vol. 53, no. 3, September 1995, pages 228-232, XP002089095 see the whole document	1-44

-/--

[X] Further documents are listed in the continuation of box C.

[X] Patent family members are listed in annex.

° Special categories of cited documents :

"A" document defining the general state of the art which is not considered to be of particular relevance
"E" earlier document but published on or after the international filing date
"L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)
"O" document referring to an oral disclosure, use, exhibition or other means
"P" document published prior to the international filing date but later than the priority date claimed

"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention
"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone
"Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art.
"&" document member of the same patent family

Date of the actual completion of the international search	Date of mailing of the international search report
5 January 1999	18/01/1999

Name and mailing address of the ISA	Authorized officer
European Patent Office, P.B. 5818 Patentlaan 2 NL - 2280 HV Rijswijk Tel. (+31-70) 340-2040, Tx. 31 651 epo nl, Fax: (+31-70) 340-3016	Oderwald, H

2

Form PCT/ISA/210 (second sheet) (July 1992)

Inte :onal Application No
PCT/US 98/14665

C.(Continuation) DOCUMENTS CONSIDERED TO BE RELEVANT		
Category °	Citation of document, with indication,where appropriate, of the relevant passages	Relevant to claim No.
P,X	MENG X-J ET AL.: "A novel virus in swine is closely related to the human hepatitis E virus" PROCEEDINGS OF THE NATIONAL ACADEMY OF SCIENCES OF THE USA, vol. 94, September 1997, pages 9860-9865, XP002088357 see the whole document -----	1-8, 10-15, 17-41

2

INTERNATIONAL SEARCH REPORT

International application No.
PCT/US 98/ 14665

Box I Observations where certain claims were found unsearchable (Continuation of item 1 of first sheet)

This International Search Report has not been established in respect of certain claims under Article 17(2)(a) for the following reasons:

1. [X] Claims Nos.:
because they relate to subject matter not required to be searched by this Authority, namely:
Remark: Although claim(s) 28 and 29 is(are) directed to a method of treatment of the human/animal body, the search has been carried out and based on the alleged effects of the compound/composition.

2. [ ] Claims Nos.:
because they relate to parts of the International Application that do not comply with the prescribed requirements to such an extent that no meaningful International Search can be carried out, specifically:

3. [ ] Claims Nos.:
because they are dependent claims and are not drafted in accordance with the second and third sentences of Rule 6.4(a).

Box II Observations where unity of invention is lacking (Continuation of item 2 of first sheet)

This International Searching Authority found multiple inventions in this international application, as follows:

1. [ ] As all required additional search fees were timely paid by the applicant, this International Search Report covers all searchable claims.

2. [ ] As all searchable claims could be searched without effort justifying an additional fee, this Authority did not invite payment of any additional fee.

3. [ ] As only some of the required additional search fees were timely paid by the applicant, this International Search Report covers only those claims for which fees were paid, specifically claims Nos.:

4. [ ] No required additional search fees were timely paid by the applicant. Consequently, this International Search Report is restricted to the invention first mentioned in the claims; it is covered by claims Nos.:

Remark on Protest

[ ] The additional search fees were accompanied by the applicant's protest.

[ ] No protest accompanied the payment of additional search fees.

INTERNATIONAL SEARCH REPORT

Information on patent family members

Inte ional Application No
PCT/US 98/14665

Patent document cited in search r port	Publication date	Patent family member(s)	Publication date
WO 9610580 A	11-04-1996	AU 3830995 A	26-04-1996
		CN 1168698 A	24-12-1997
		EP 0784631 A	23-07-1997
		NO 971529 A	26-05-1997